COPY

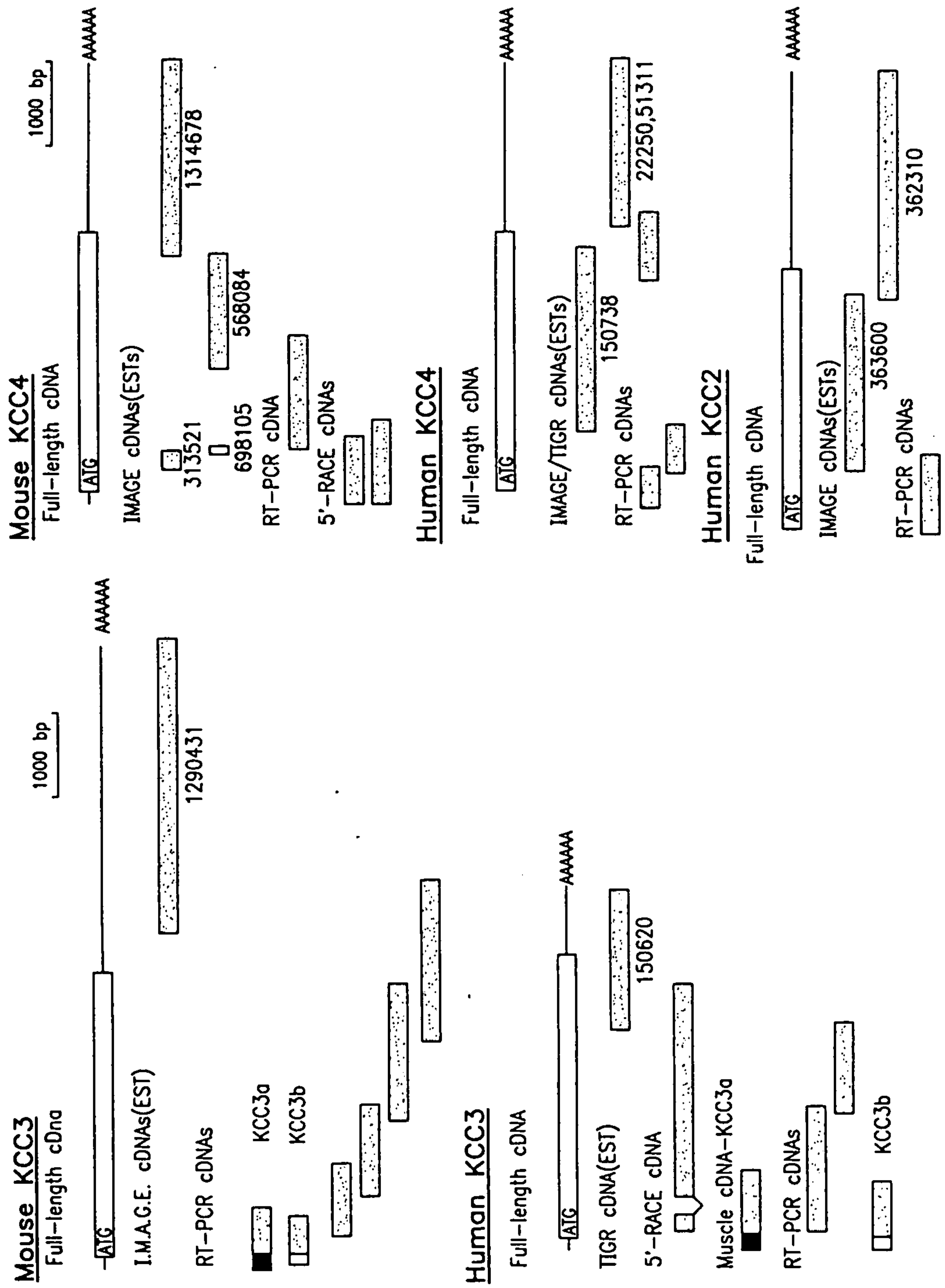

Mouse KCC3
Full-length cDna
ATG
AAAAA
1000 bp
I.M.A.G.E. cDNAs(EST)
1290431
RT-PCR cDNAs
KCC3a
KCC3b
Human KCC3
Full-length cDNA
ATG
AAAAA
TIGR cDNA(EST)
150620
5'-RACE cDNA
Muscle cDNA-KCC3a
RT-PCR cDNAs
KCC3b
Mouse KCC4
Full-length cDNA
ATG
AAAAA
1000 bp
IMAGE cDNAs(ESTs)
313521
698105
568084
1314678
RT-PCR cDNA
5'-RACE cDNAs
Human KCC4
Full-length cDNA
ATG
AAAAA
IMAGE/TIGR cDNAs(ESTs)
150738
22250,51311
RT-PCR cDNAs
Human KCC2
Full-length cDNA
ATG
AAAAA
IMAGE cDNAs(ESTs)
363600
362310
RT-PCR cDNAs

FIG. 1

FIG. 2A

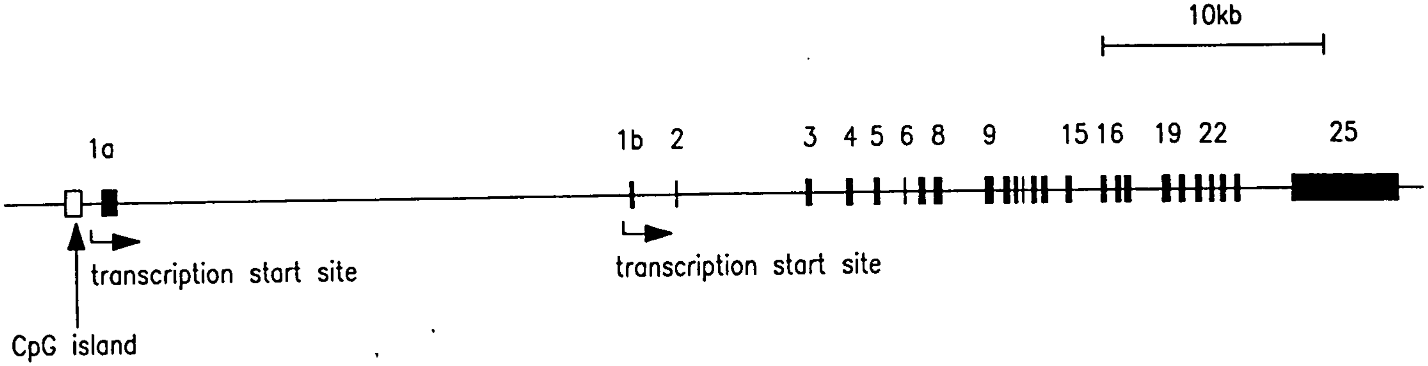
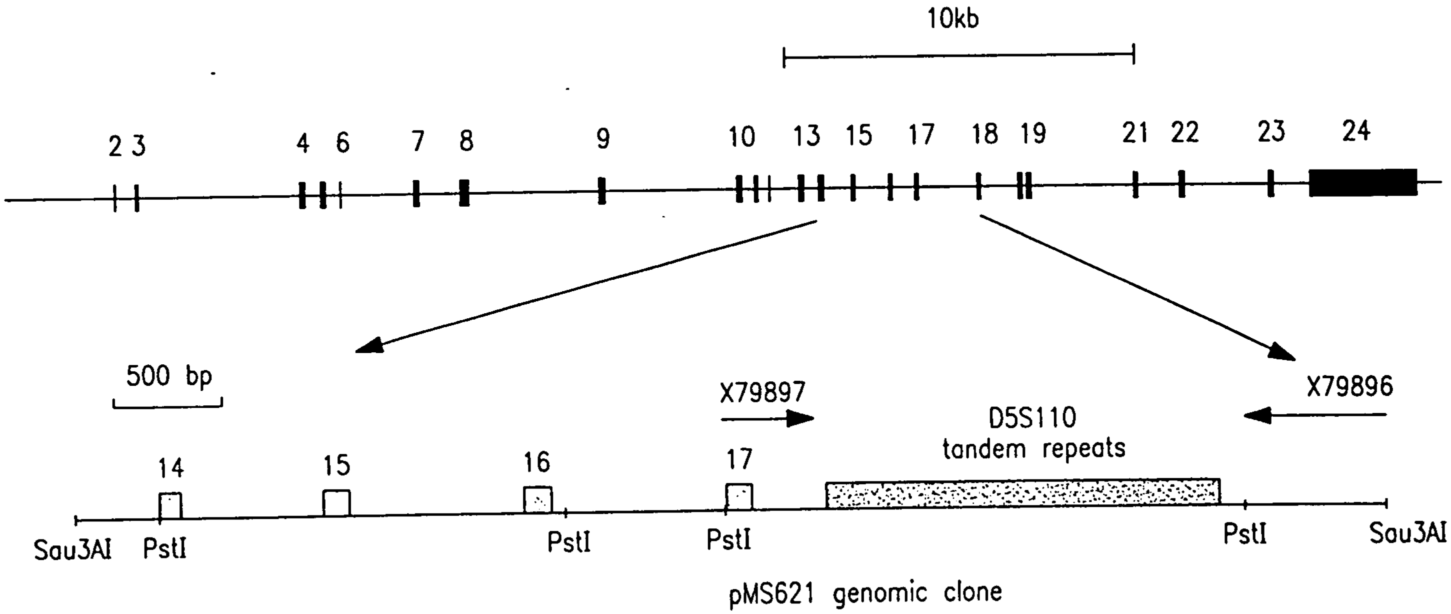

10kb
1a
1b
2
3
4
5
6
8
9
15
16
19
22
25
transcription start site
transcription start site
CpG island

FIG. 2B


10kb
2 3
4
6
7
8
9
10
13
15
17
18
19
21
22
23
24
500 bp
X79897
X79896
D5S110
tandem repeats
14
15
16
17
Sau3AI
PstI
PstI
PstI
PstI
Sau3AI
pMS621 genomic clone

Title: Purified and Isolated Potassium-Chloride Cotransporter Nu[illegible]ic Acids and Polypeptides and Therapeutic and [illegible]ing Methods Using Same
Applicant: Mount et al.
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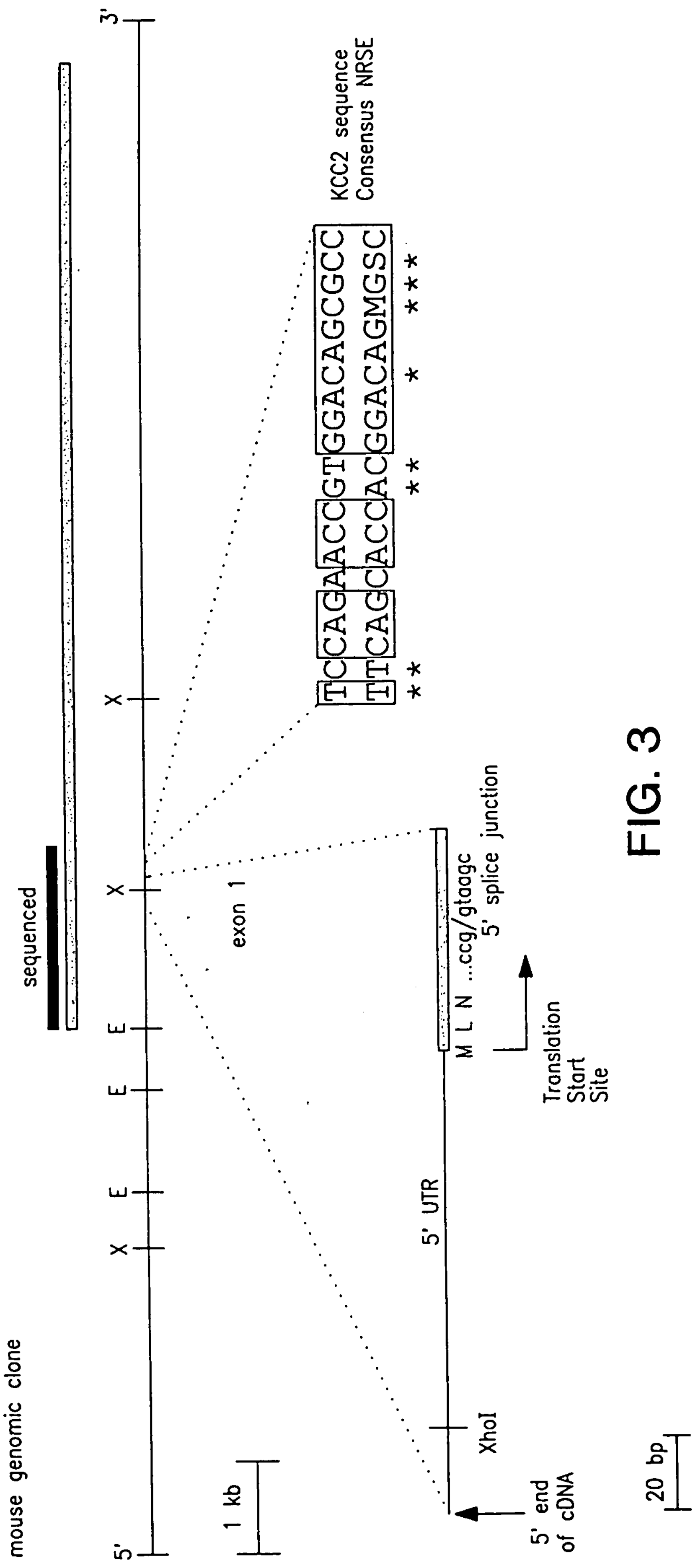


FIG. 3

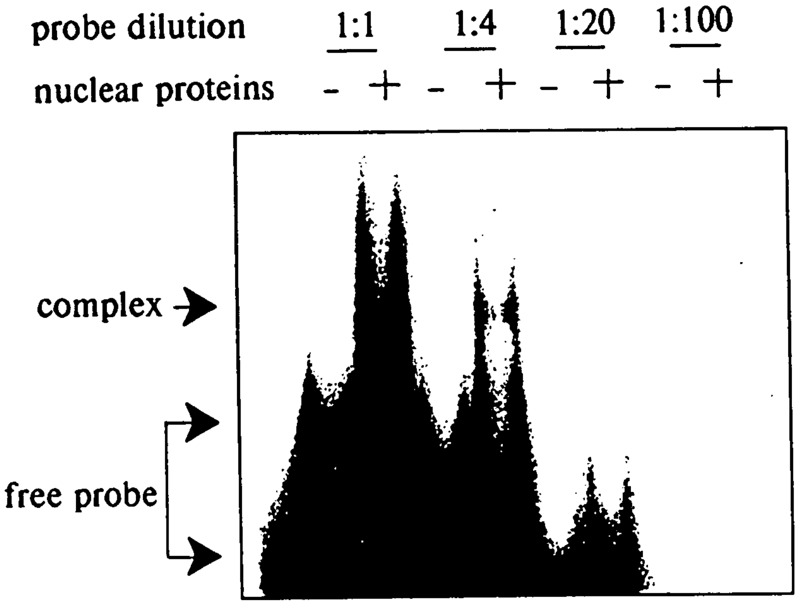

probe dilution
1:1
1:4
1:20
1:100
nuclear proteins
- + - + - + - +
complex
free probe

FIG. 4A

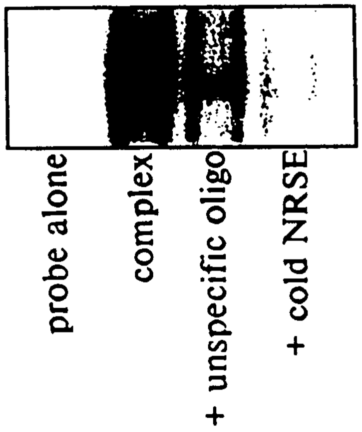

probe alone
complex
+ unspecific oligo
+ cold NRSE

FIG. 4B

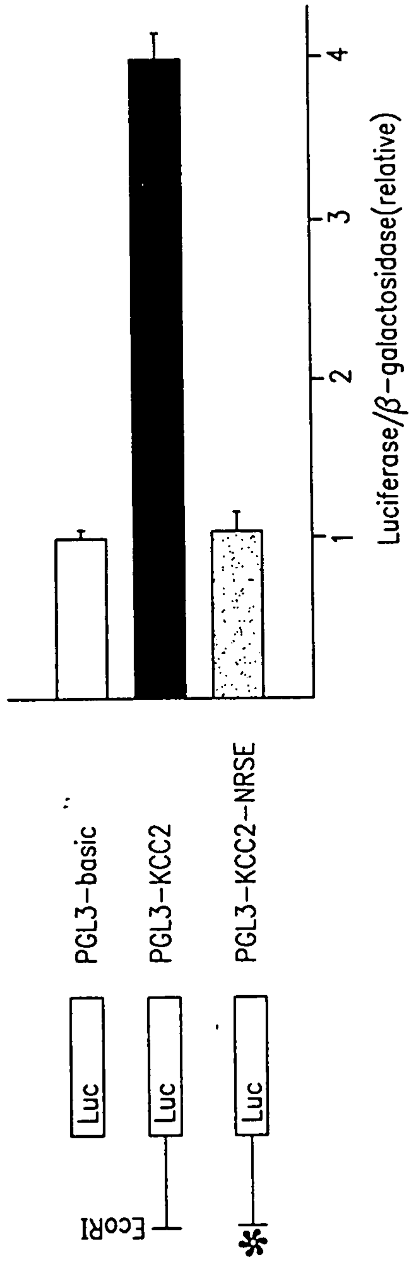

Luc
Luc
Luc
EcoRI
*
PGL3-basic
PGL3-KCC2
PGL3-KCC2-NRSE
1
2
3
4
Luciferase/β-galactosidase(relative)

FIG. 5

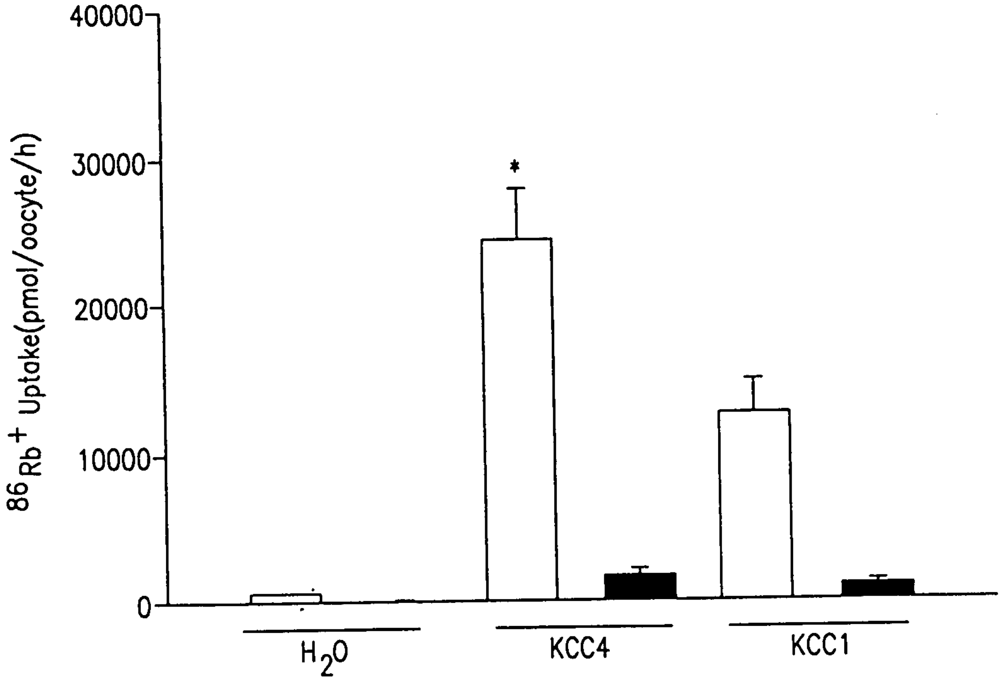

40000
30000
20000
10000
0
$^{86}Rb^+$ Uptake(pmol/oocyte/h)
*
H_2O
KCC4
KCC1

FIG. 6

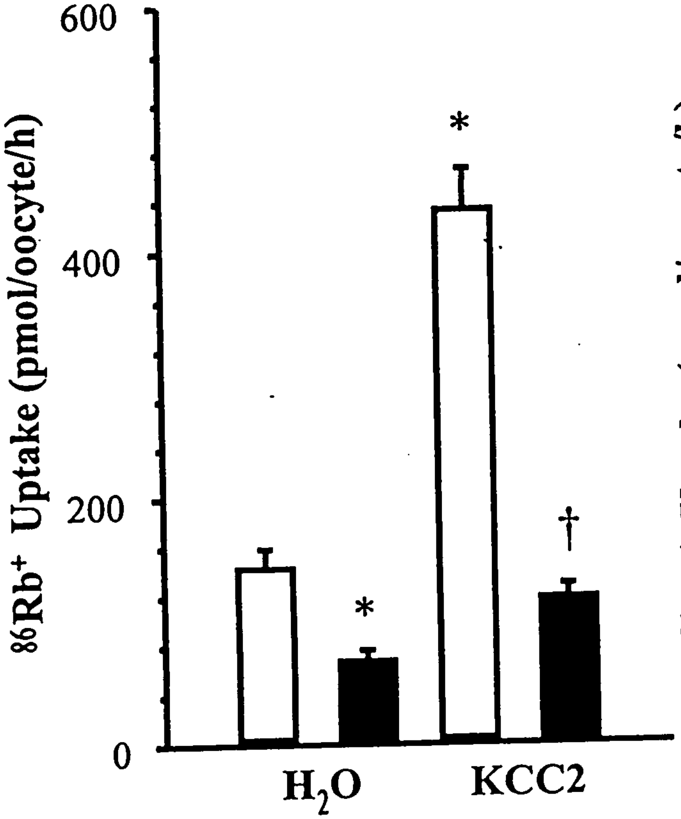

600
400
200
0
$^{86}Rb^+$ Uptake (pmol/oocyte/h)
*
*
†
H_2O
KCC2

FIG. 7A

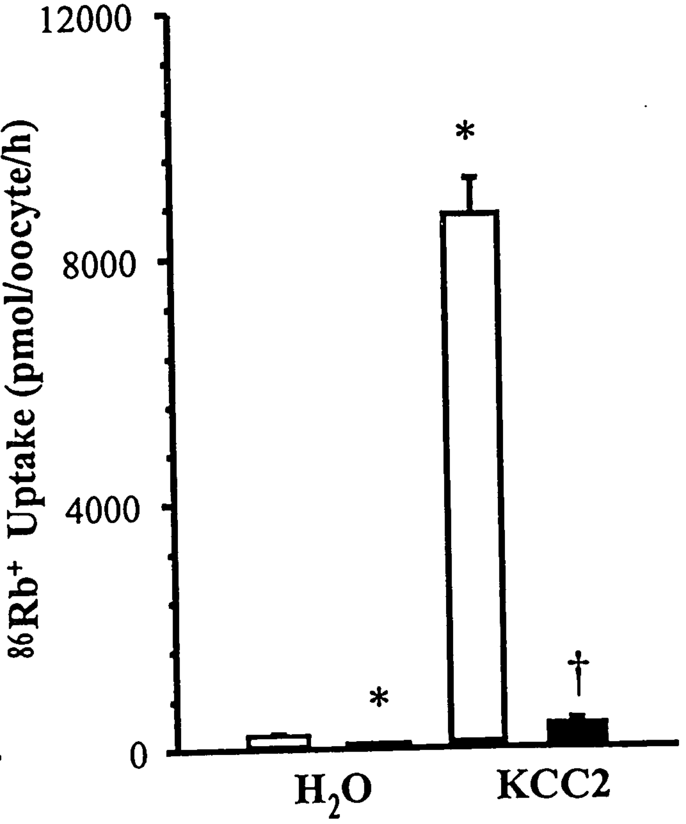

12000
8000
4000
0
$^{86}Rb^+$ Uptake (pmol/oocyte/h)
*
*
†
H_2O
KCC2

FIG. 7B

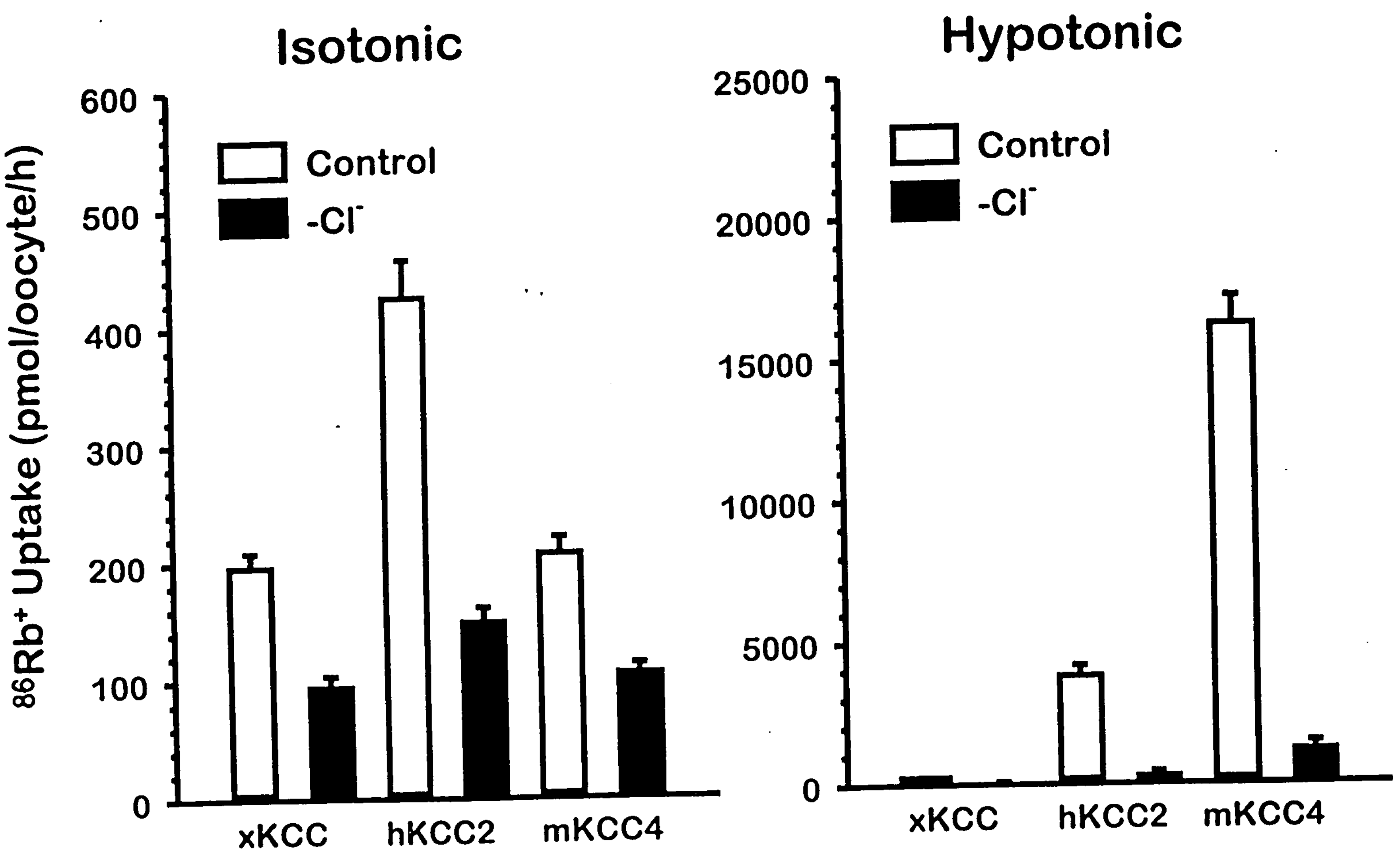

Isotonic
Hypotonic
Control
-Cl⁻
Control
-Cl⁻
86Rb+ Uptake (pmol/oocyte/h)
600
500
400
300
200
100
0
25000
20000
15000
10000
5000
0
xKCC
hKCC2
mKCC4
xKCC
hKCC2
mKCC4

FIG. 8

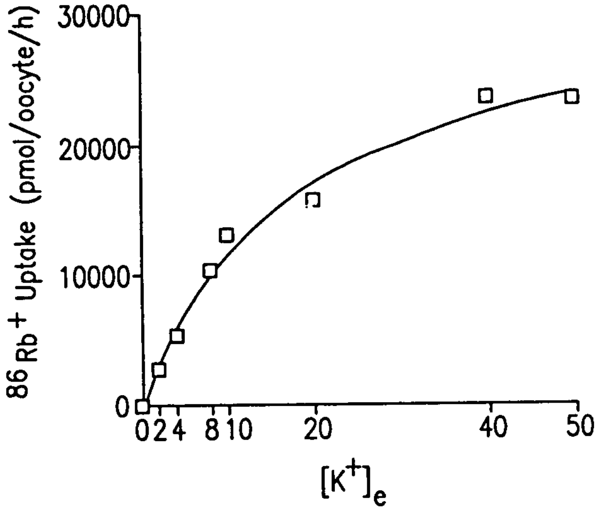


FIG. 9A

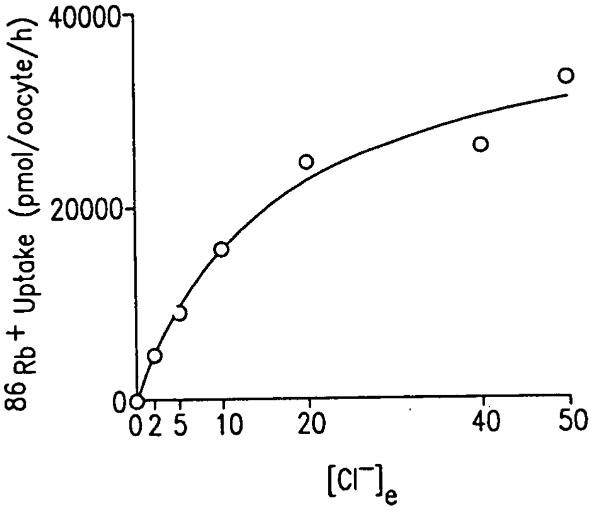


FIG. 9B

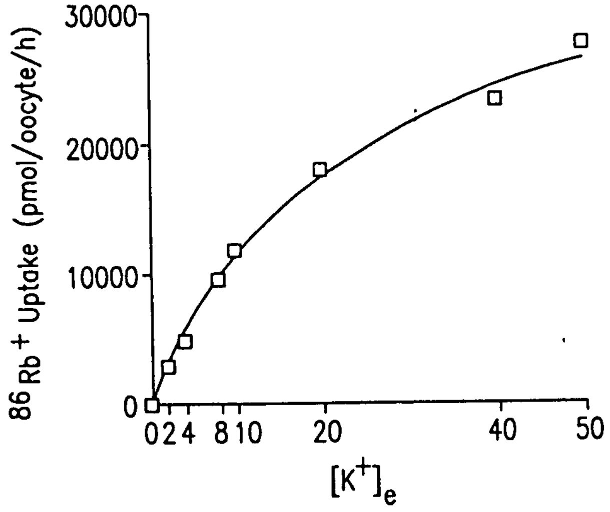


FIG. 9C

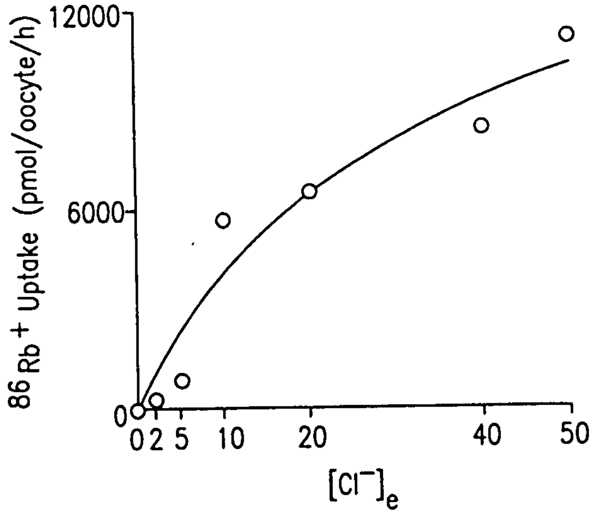


FIG. 9D

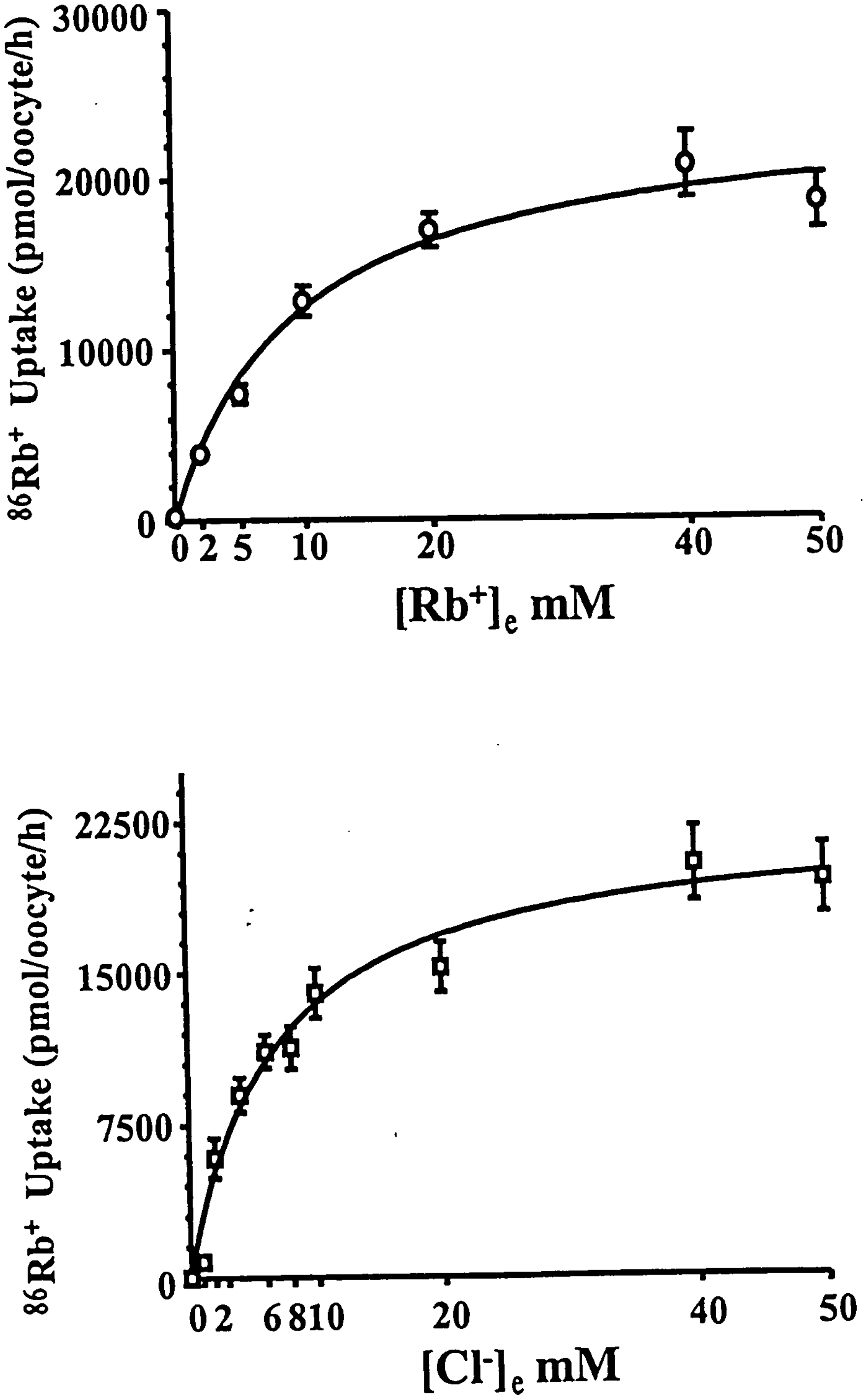

30000
20000
10000
0
86Rb+ Uptake (pmol/oocyte/h)
0 2 5 10 20 40 50
[Rb+]e mM
22500
15000
7500
0
86Rb+ Uptake (pmol/oocyte/h)
0 2 6 8 10 20 40 50
[Cl-]e mM

FIG. 10

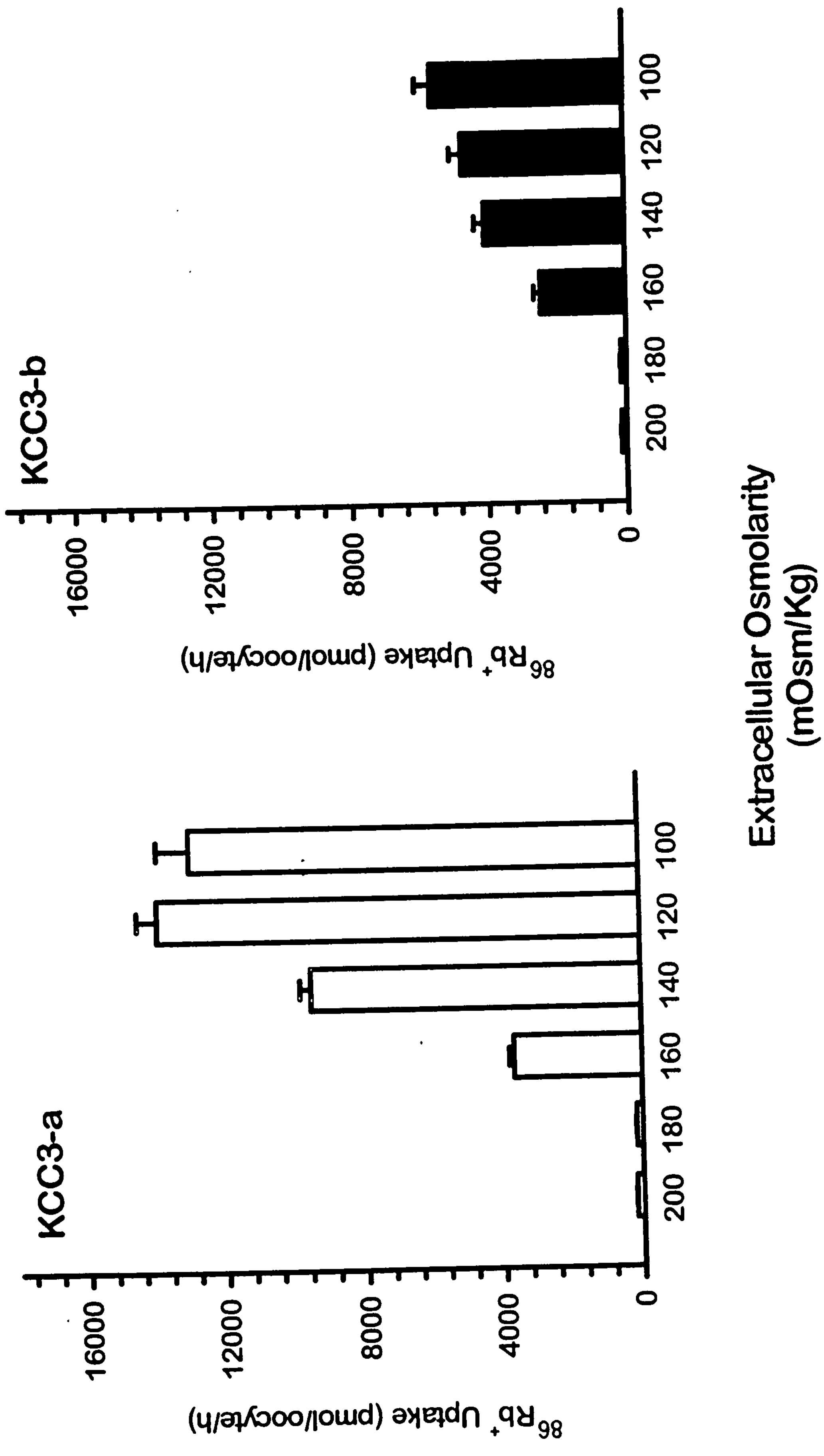

KCC3-a
KCC3-b
$^{86}Rb^+$ Uptake (pmol/oocyte/h)
16000
12000
8000
4000
0
200
180
160
140
120
100
Extracellular Osmolarity (mOsm/Kg)

FIG. 11

COPY

09835976 . 030402

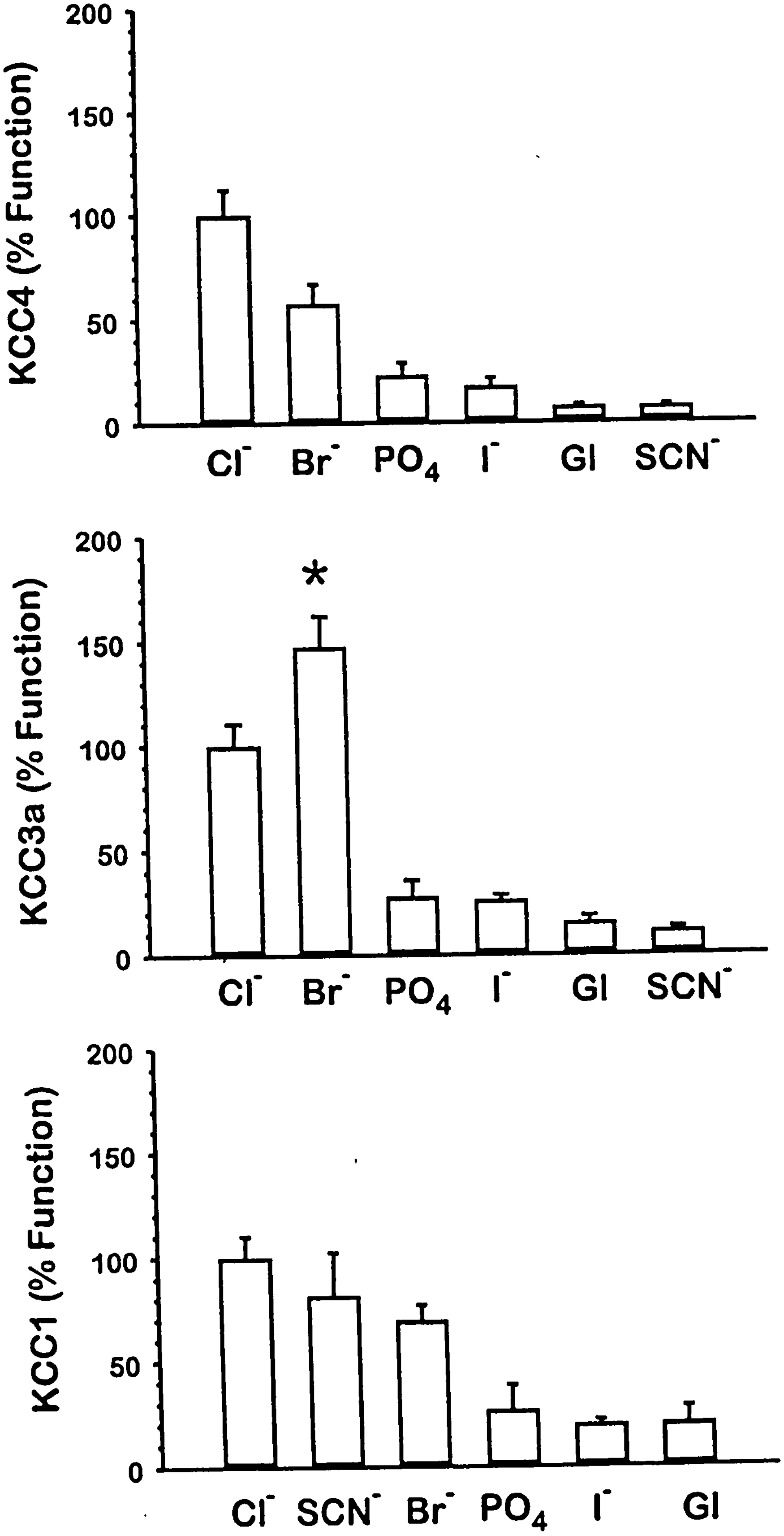


FIG. 12

Title: Purified and Isolated Potassium-Chloride Cotransporter Nucleic Acids and Polypeptides and Thr eutic and Screening Methods Using Same
licant(s): Mount et al.
Serial No.: 09/835,976

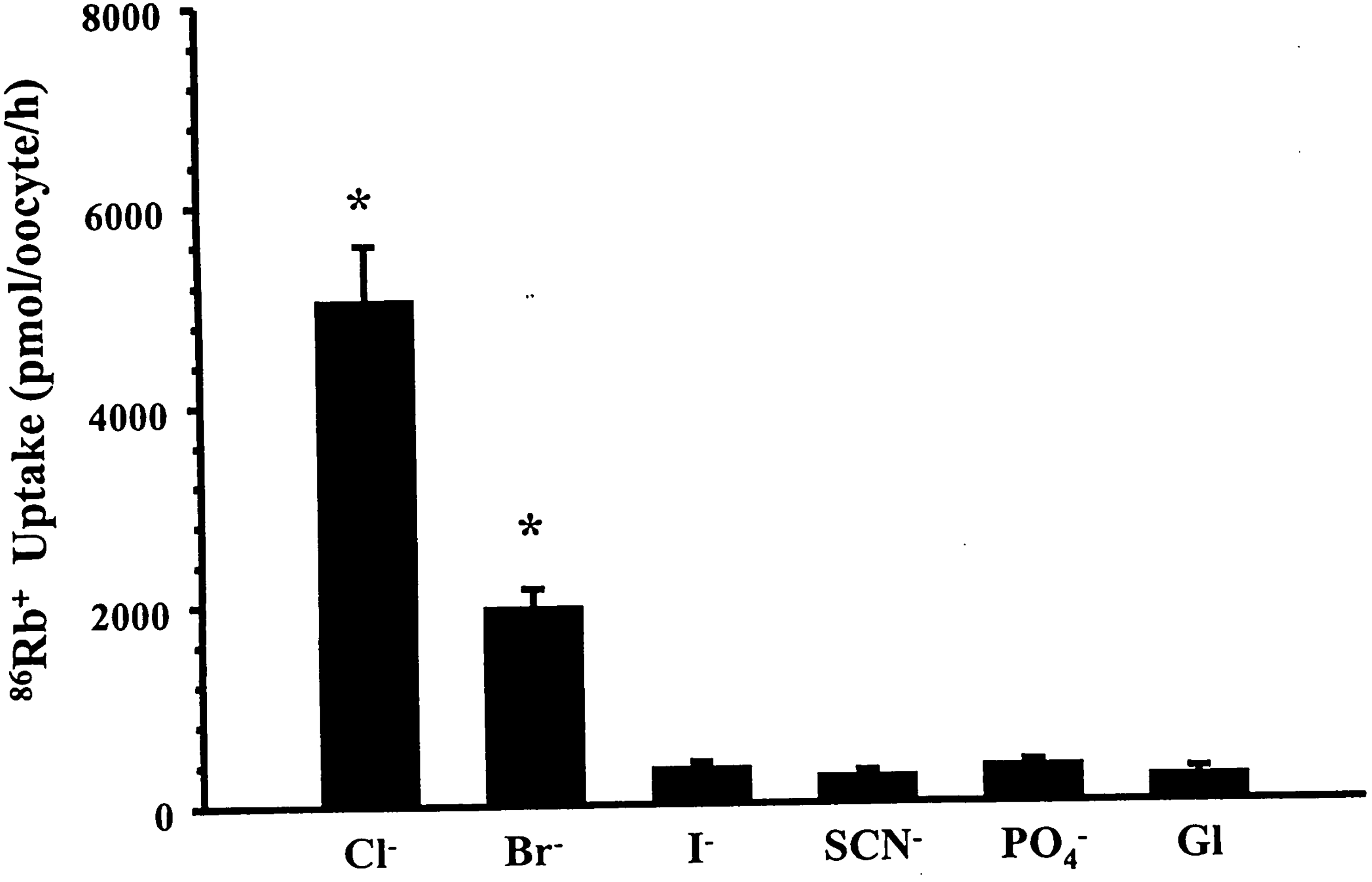


FIG. 13

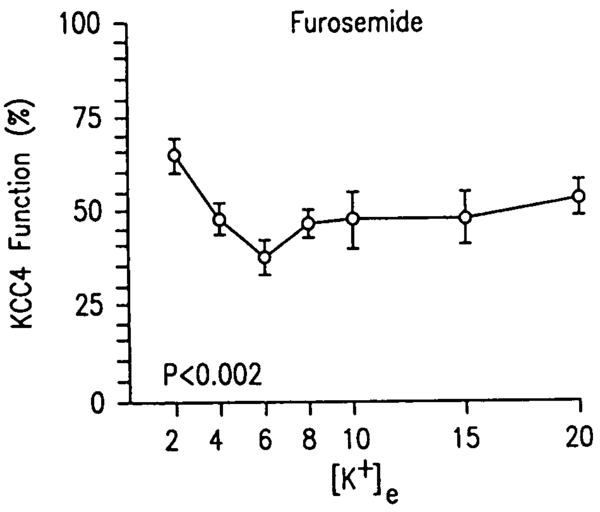


FIG. 14A

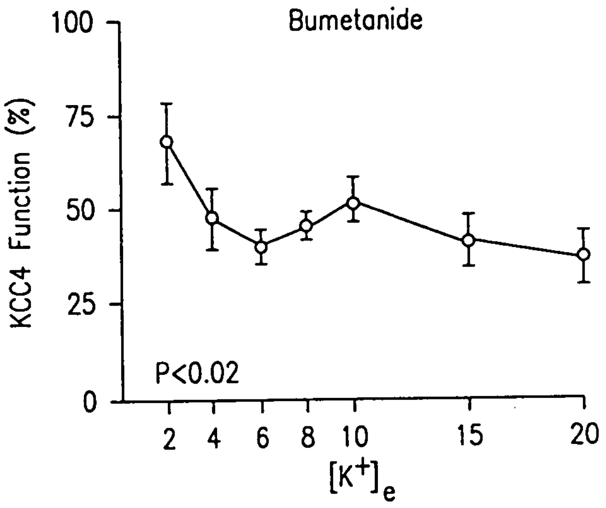


FIG. 14B

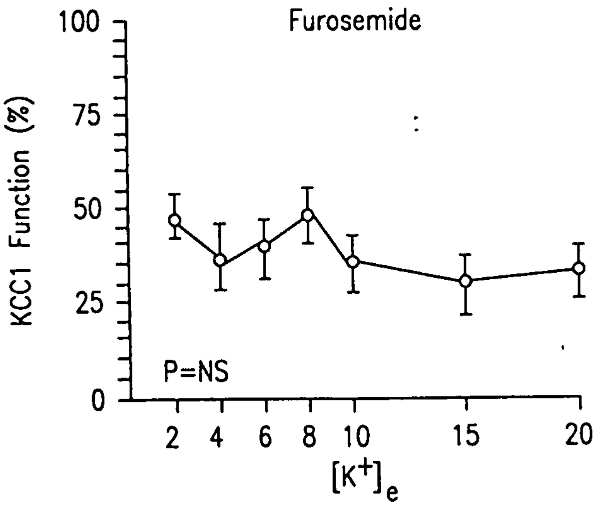


FIG. 14C

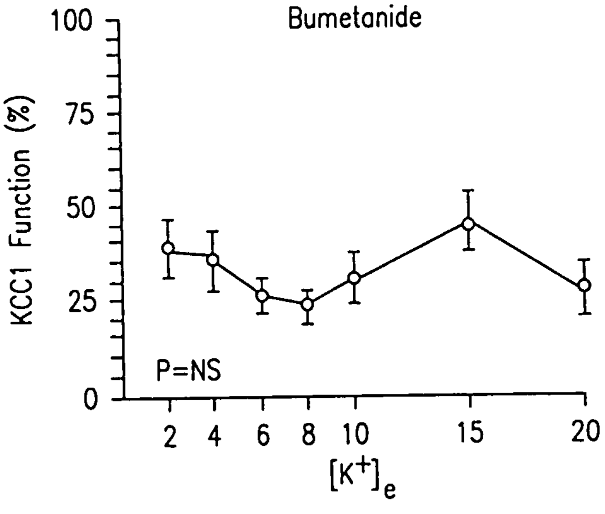


FIG. 14D

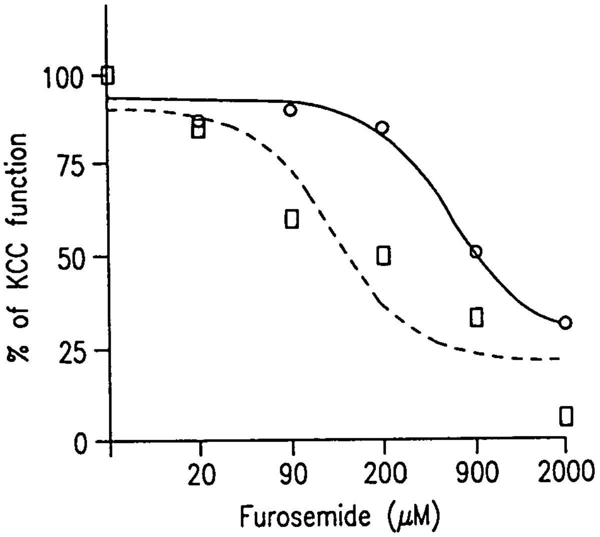


FIG. 15A

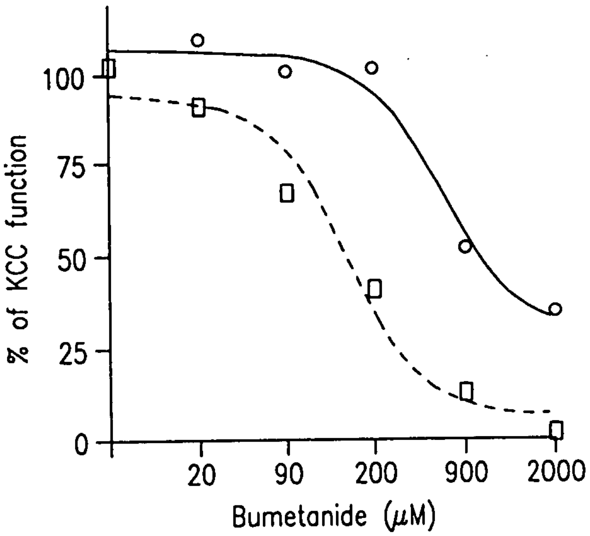


FIG. 15B

Title: Purified and Isolated Potassium-Chloride Cotransporter Nucleic Acids and Polypeptides and Therapeutic and Screening Methods Using Same
App (s): Mount et al.
Serial No.: 09/835,976

COPY

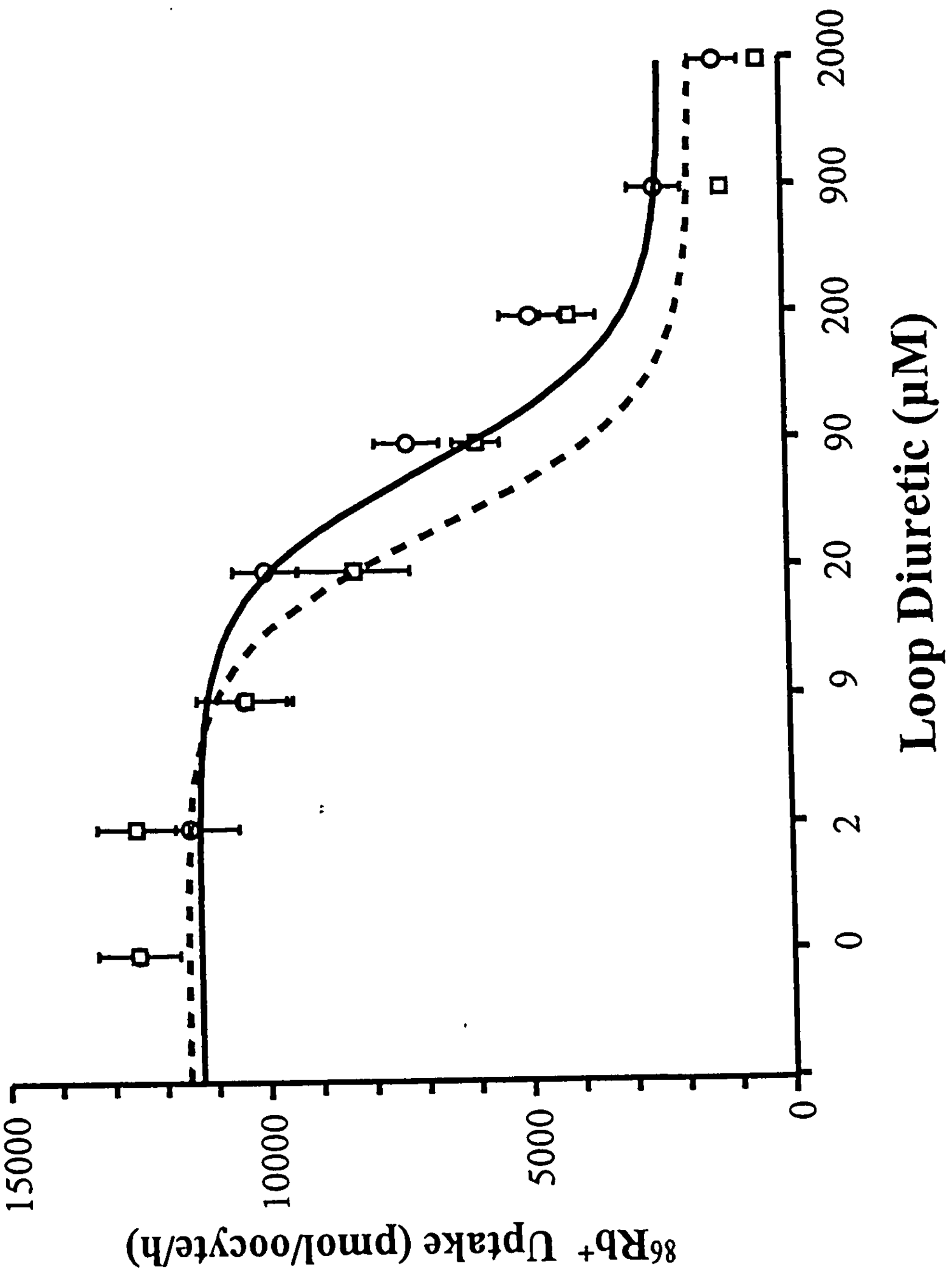


FIG. 16

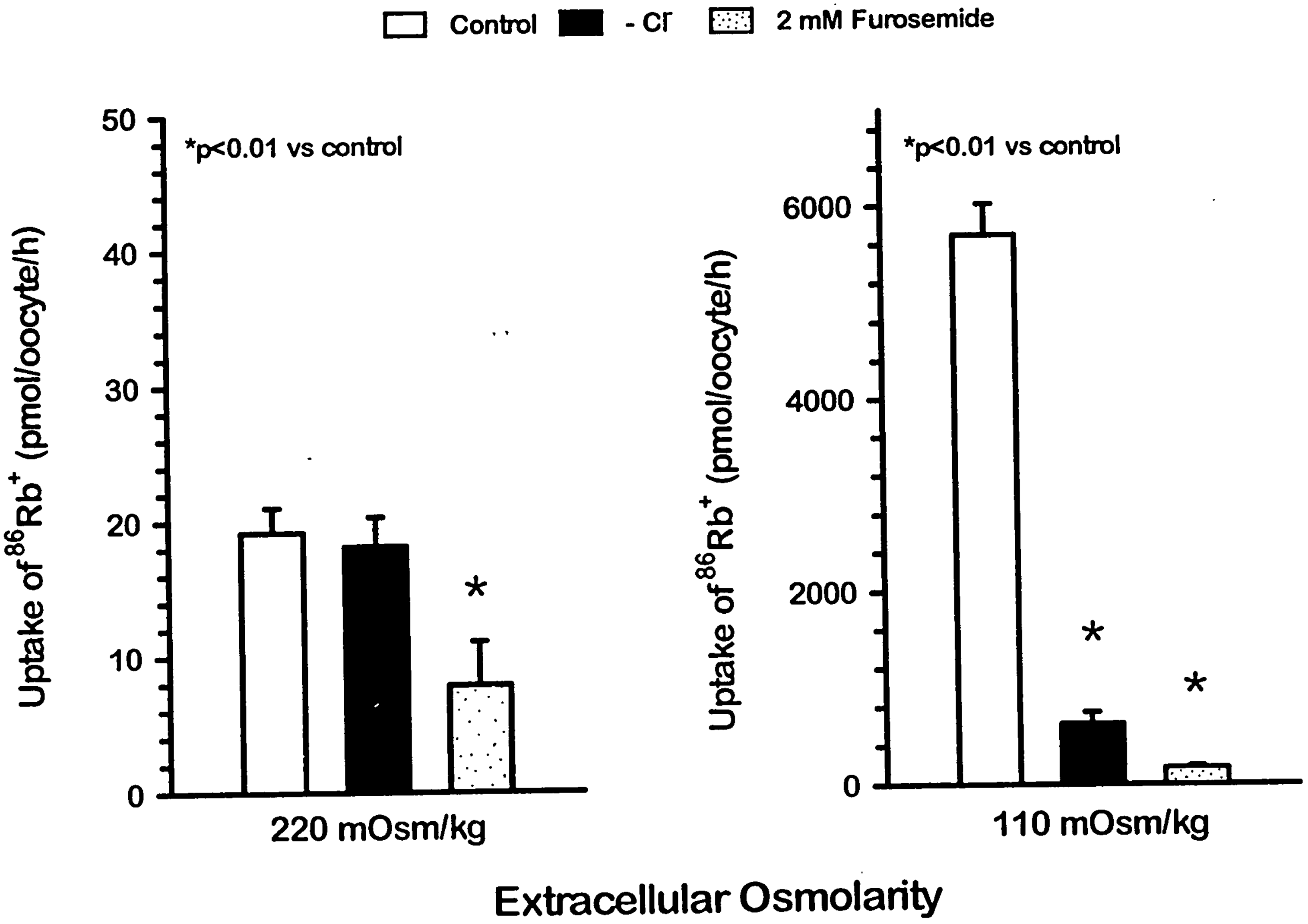

☐ Control
■ - Cl⁻
▨ 2 mM Furosemide
Uptake of 86Rb+ (pmol/oocyte/h)
50
40
30
20
10
0
*p<0.01 vs control
*
220 mOsm/kg
Uptake of 86Rb+ (pmol/oocyte/h)
6000
4000
2000
0
*p<0.01 vs control
*
*
110 mOsm/kg
Extracellular Osmolarity

FIG. 17

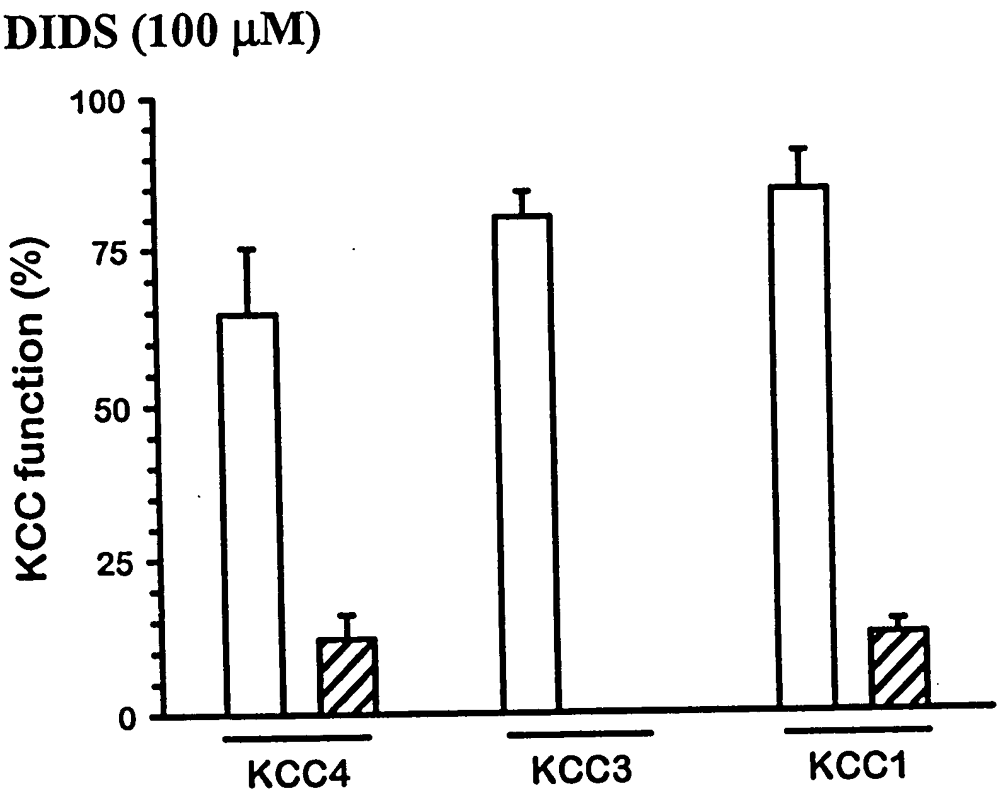


FIG. 18A

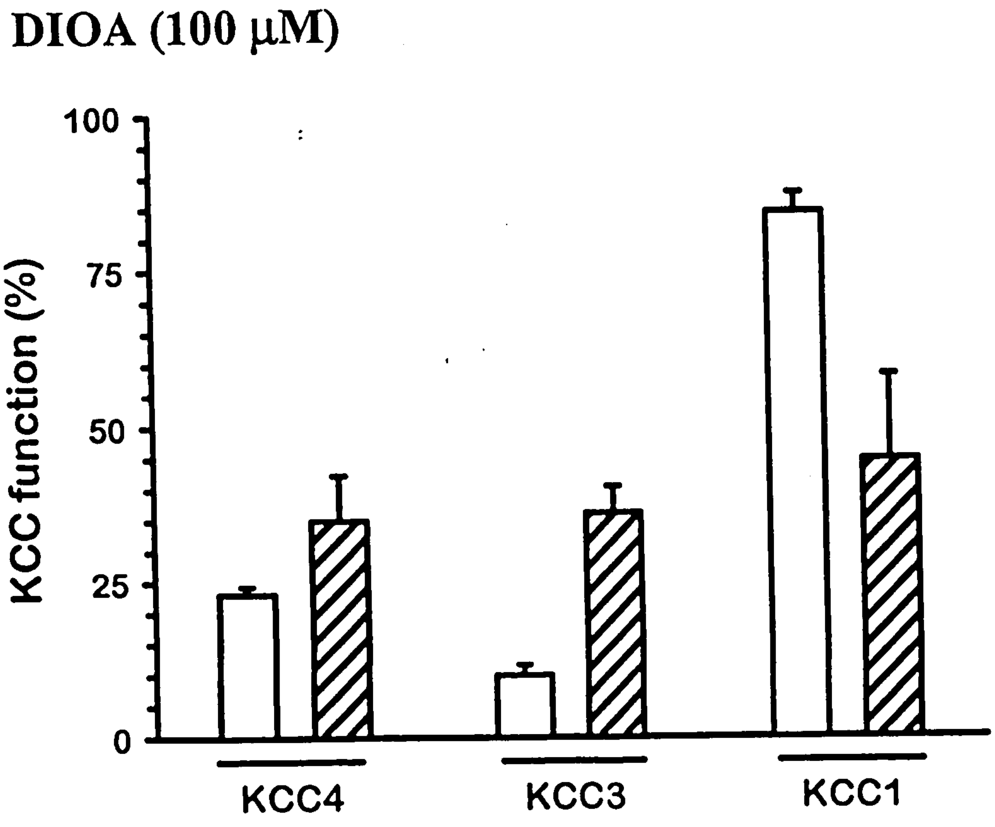


FIG. 18B

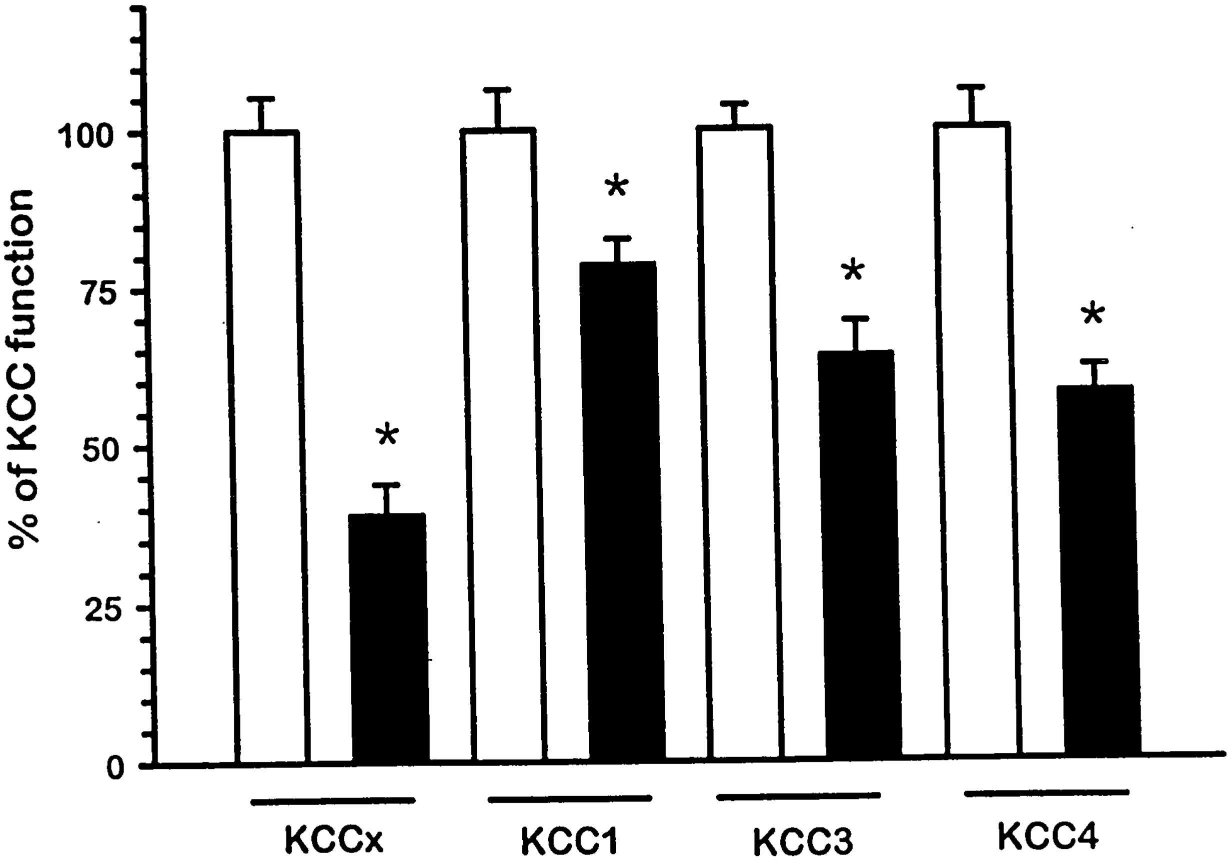


FIG. 19

Title: Purified and Isolated Potassium-Chloride Cotransporter [illegible]ucleic Acids and Polypeptides and Therapeut[illegible] and [illegible]creening Methods Using Same
Appl[illegible]s): Mount et al.
Serial No.: 09/835,976

COPY

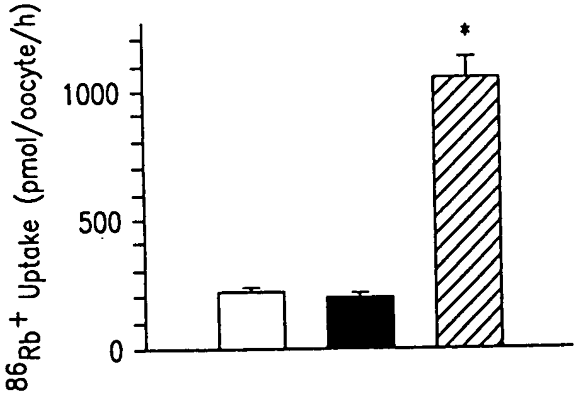


FIG. 20A

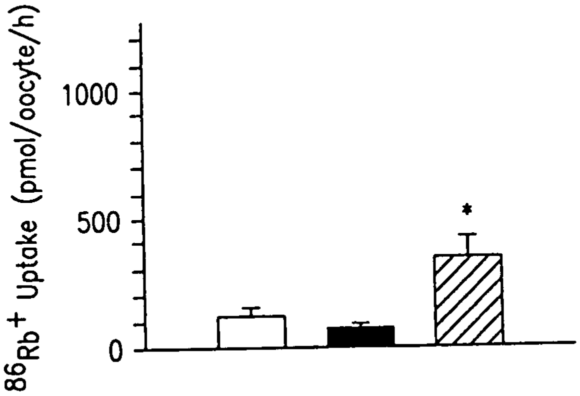


FIG. 20B

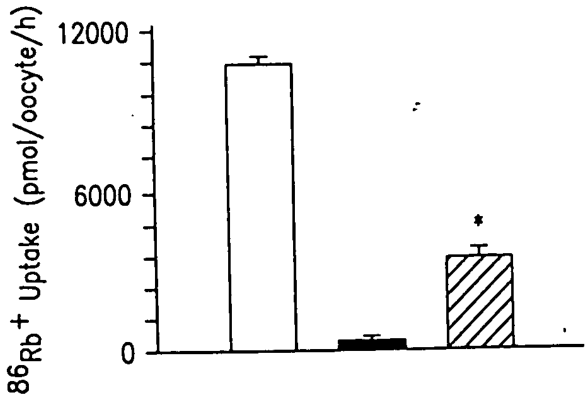


FIG. 20C

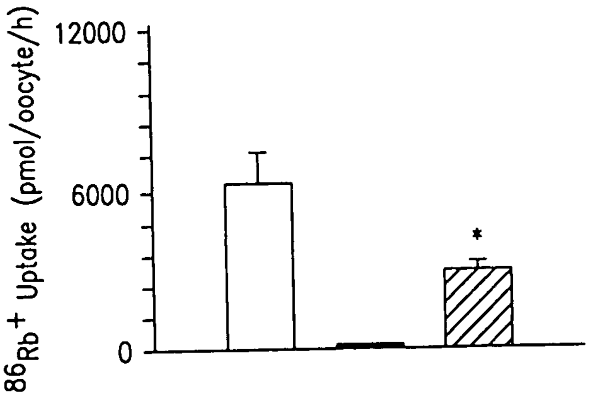


FIG. 20D

Title: Purified and Isolated Potassium-Chloride Cotransporter cleic Acids and Polypeptides and Therapeutic nd eening Methods Using Same
Applic : Mount et al.
Serial No.: 09/835,976

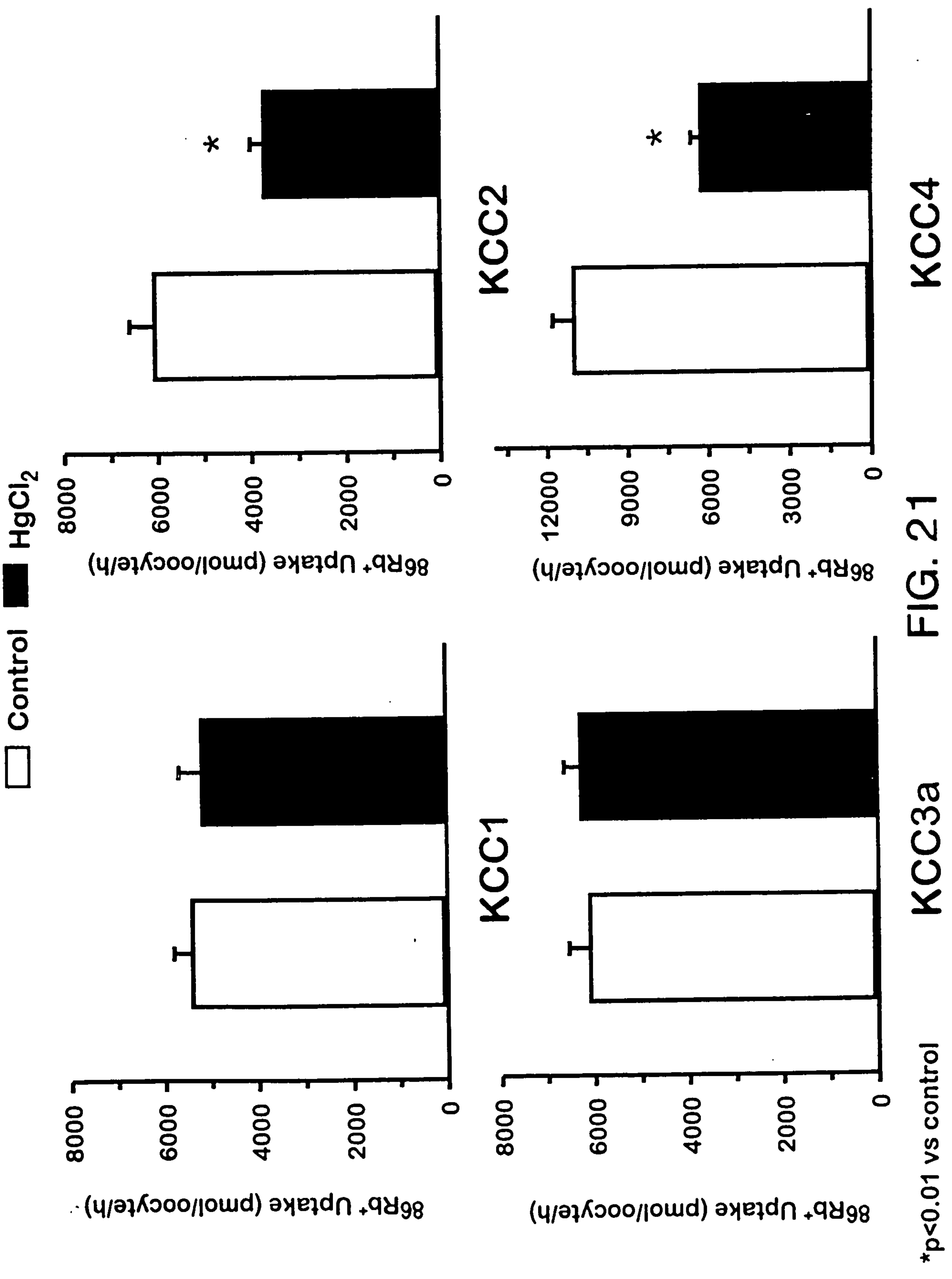
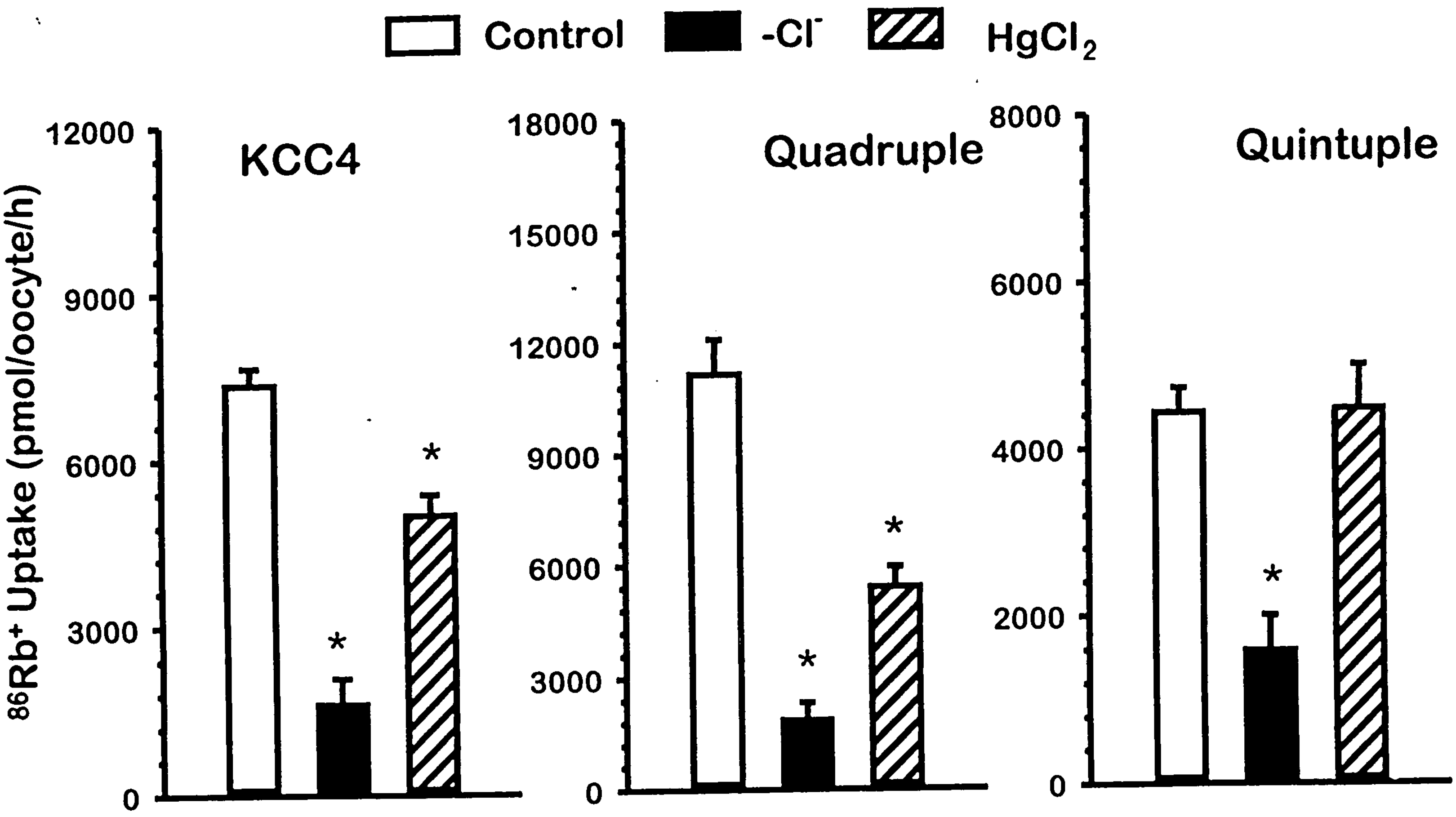


FIG. 21

Title: Purified and Isolated Potassium-Chloride Cotransporter Nucleic Acids and Polypeptides and Therapeutic and Screening Methods Using Same
Applicant(s): Mount et al.
Serial No.: 09/835,976

COPY



*p<0.01 vs control

FIG. 22

COPY

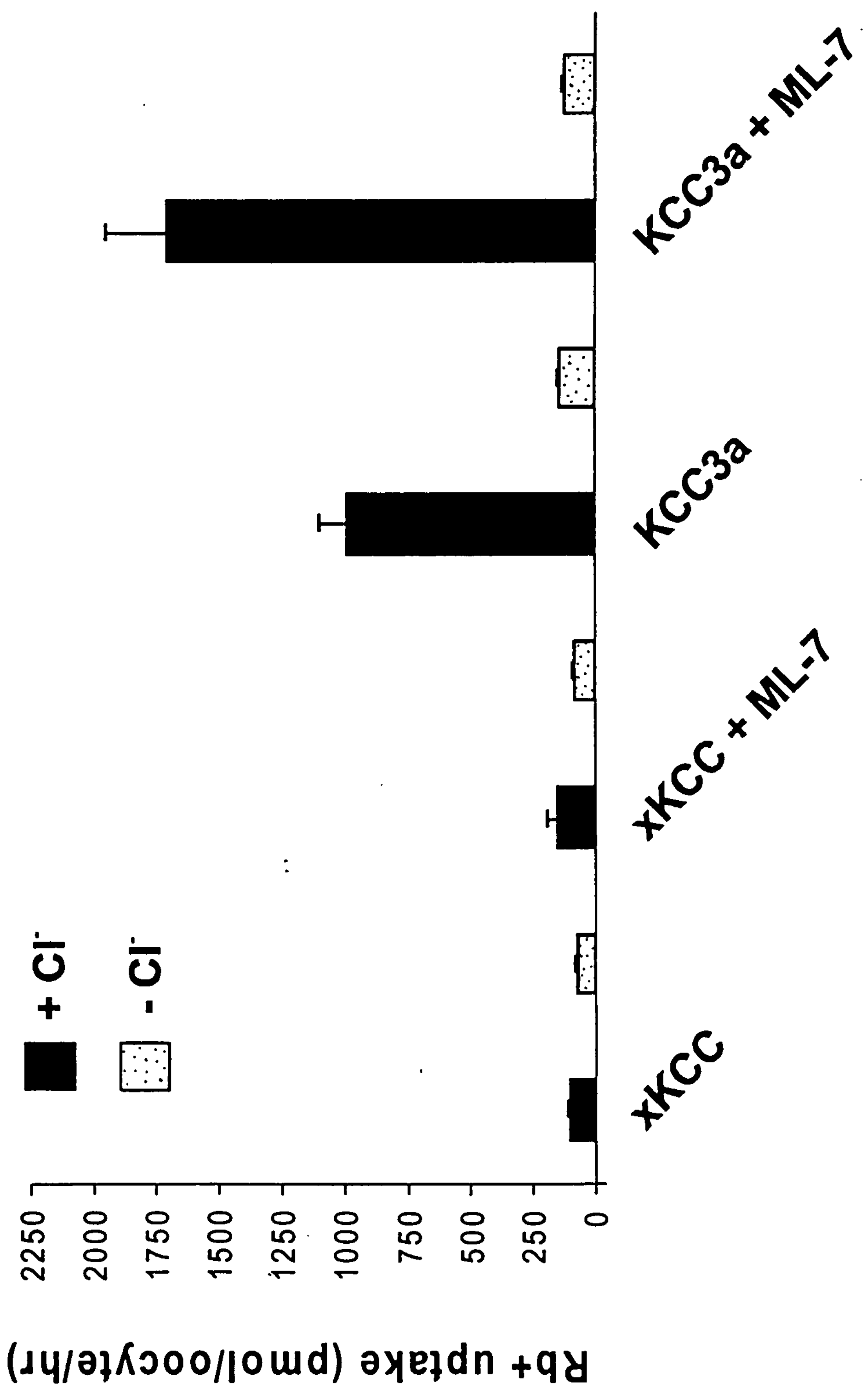

■ + Cl⁻
▨ - Cl⁻
Rb+ uptake (pmol/oocyte/hr)
2250
2000
1750
1500
1250
1000
750
500
250
0
xKCC
xKCC + ML-7
KCC3a
KCC3a + ML-7

FIG. 23

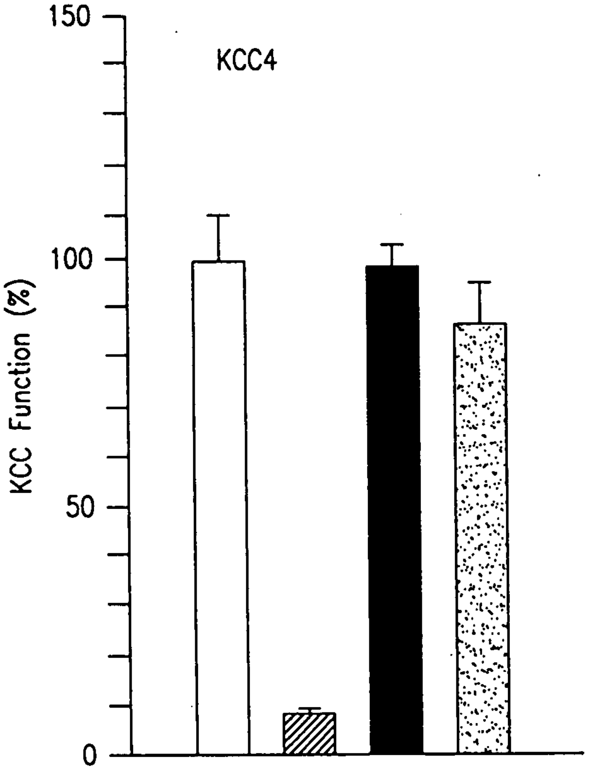


FIG. 24A

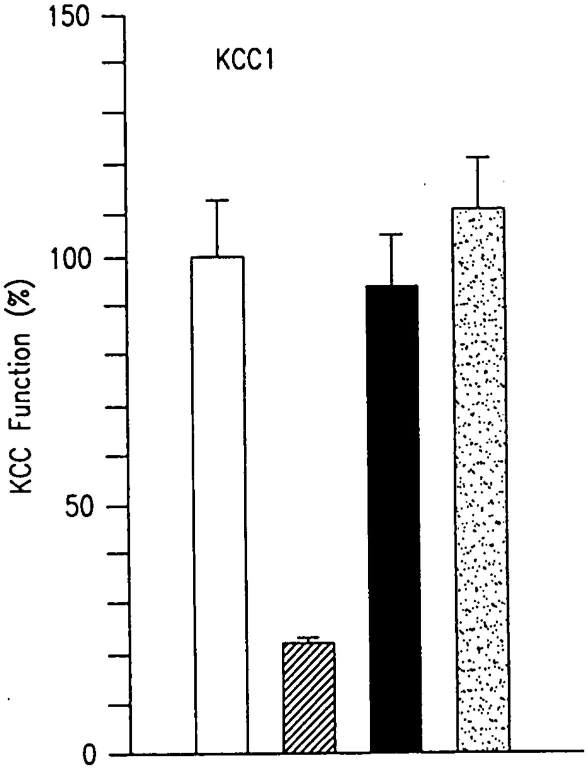


FIG. 24B

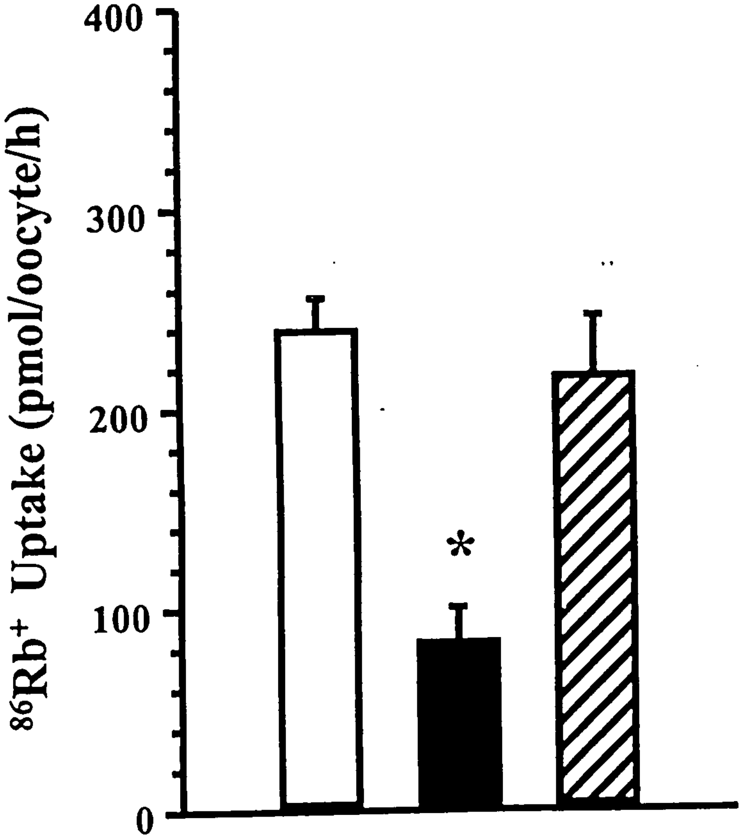


FIG. 25A

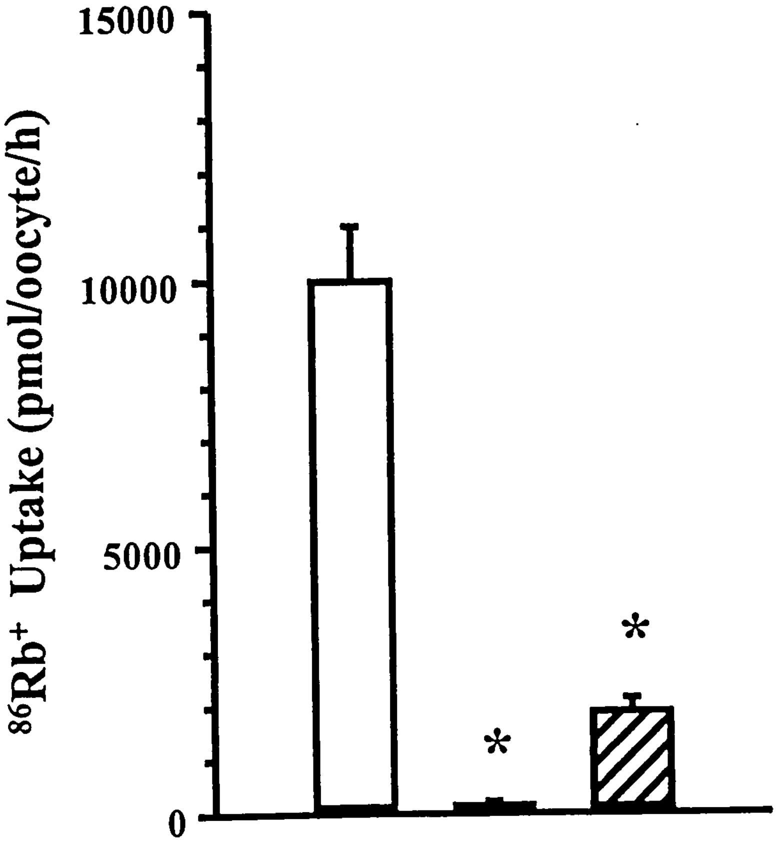


FIG. 25B

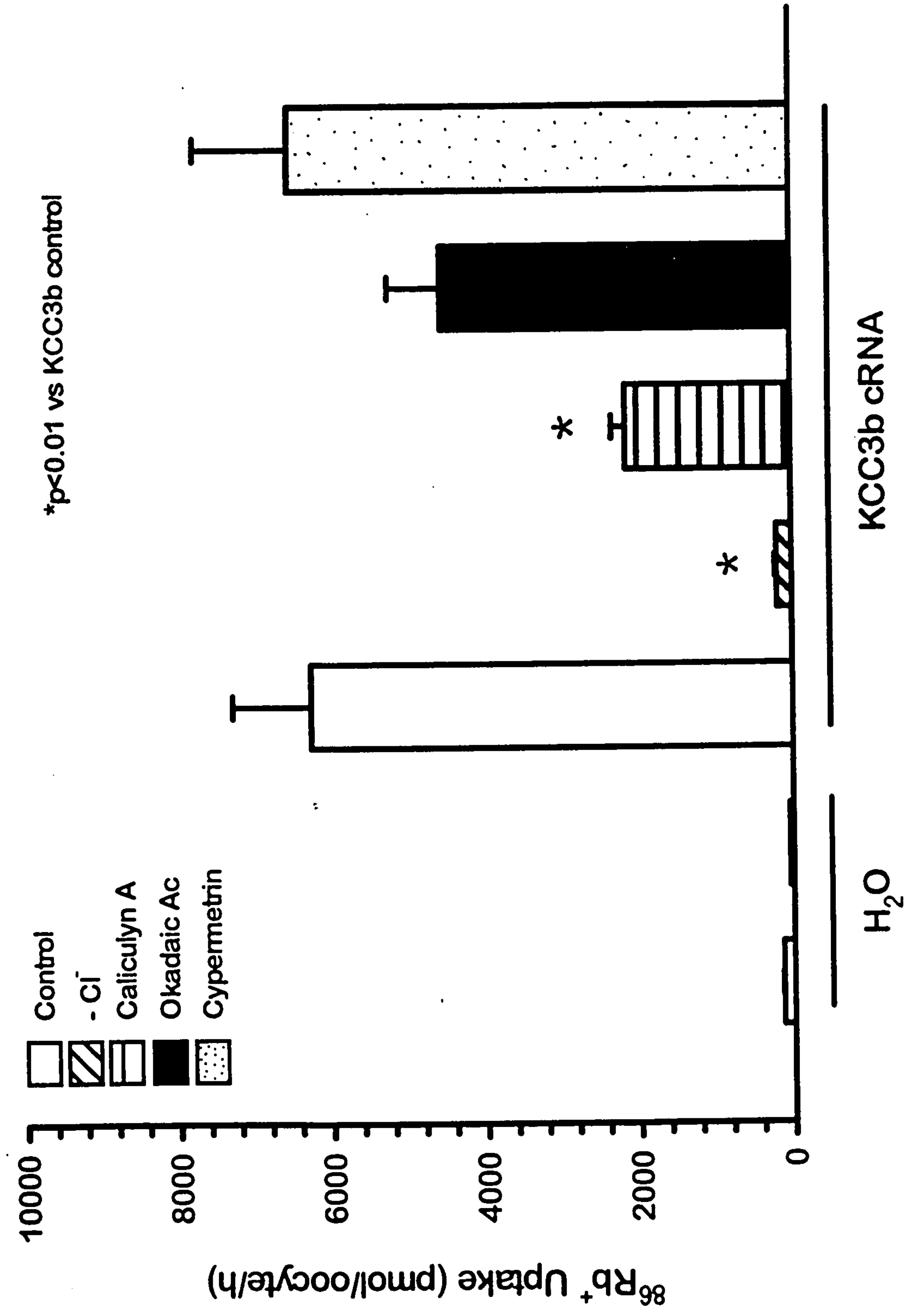

*p<0.01 vs KCC3b control
Control
- Cl⁻
Caliculyn A
Okadaic Ac
Cypermetrin
10000
8000
6000
4000
2000
0
⁸⁶Rb⁺ Uptake (pmol/oocyte/h)
*
*
H2O
KCC3b cRNA

FIG. 26

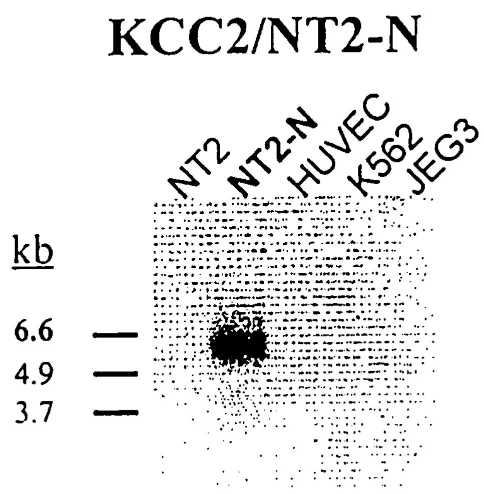


FIG. 27A

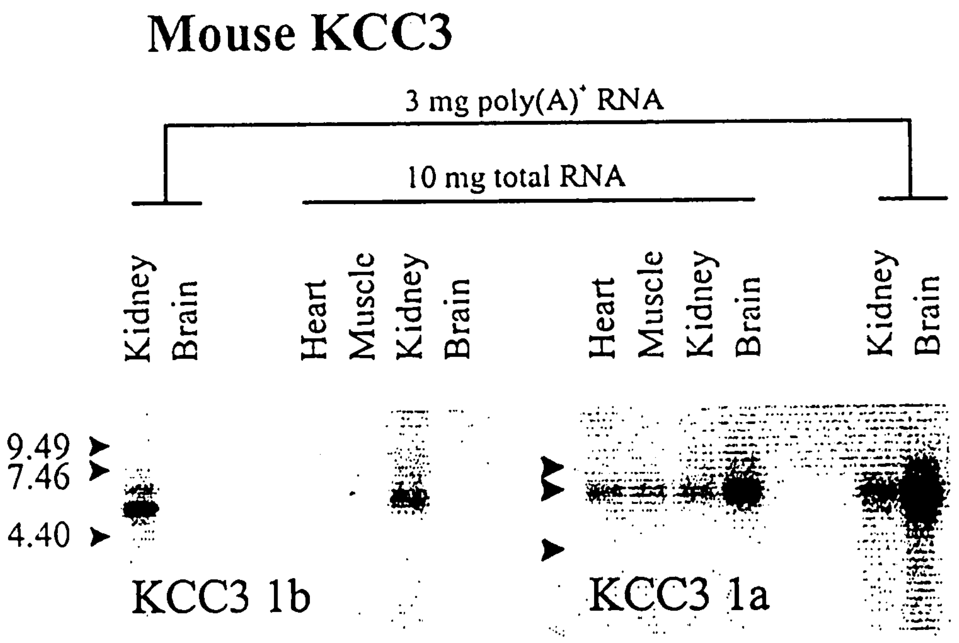


FIG. 27B

FIG. 27C

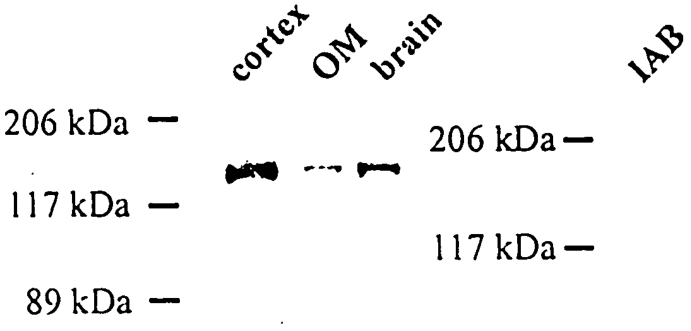


FIG. 27D

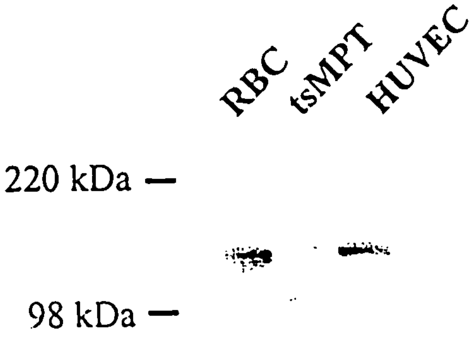


FIG. 27E

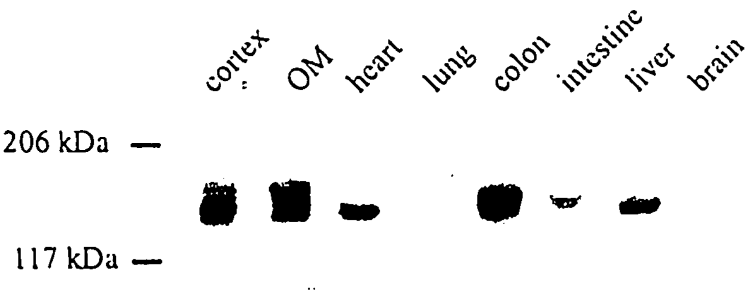


FIG. 27F

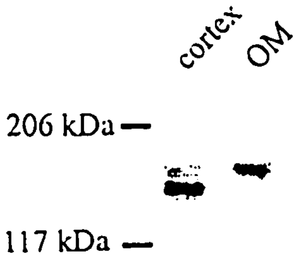


FIG. 27G

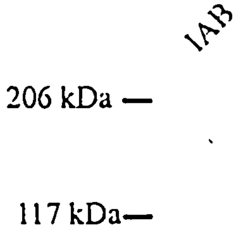


FIG. 27H

COPY

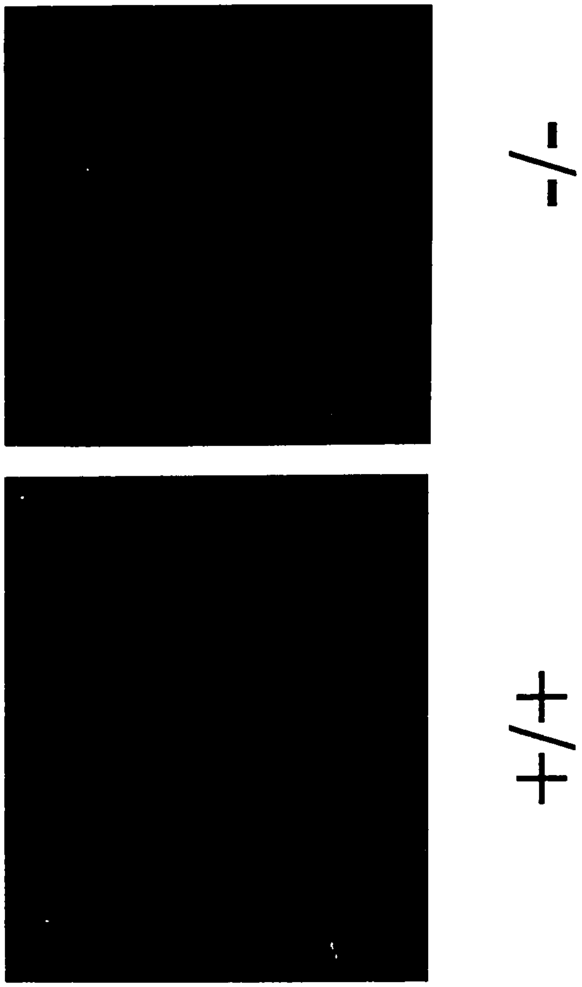


FIG. 27J

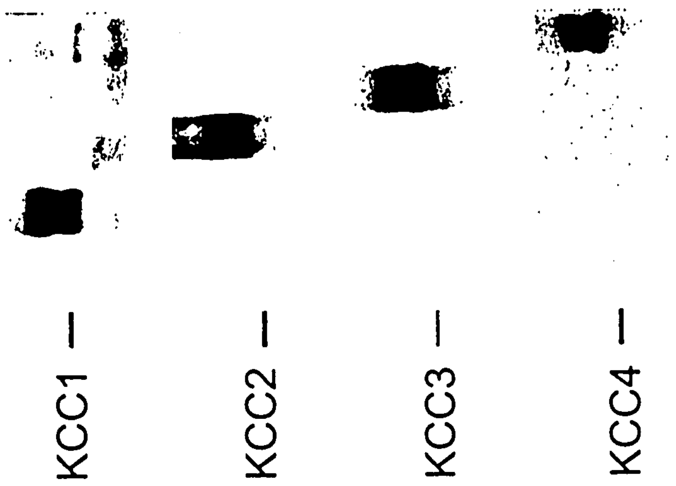


FIG. 27I

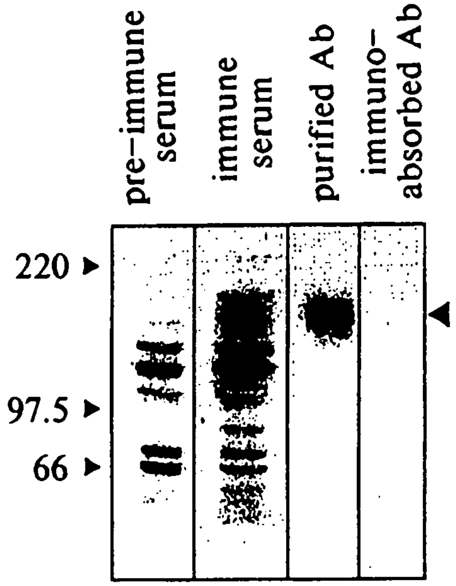

pre–immune serum
immune serum
purified Ab
immuno-absorbed Ab
220
97.5
66

FIG. 28

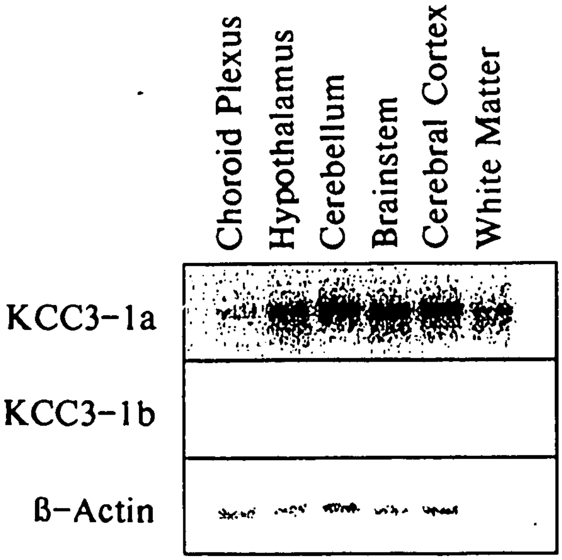

Choroid Plexus
Hypothalamus
Cerebellum
Brainstem
Cerebral Cortex
White Matter
KCC3–1a
KCC3–1b
ß–Actin

FIG. 29

FIG. 30A

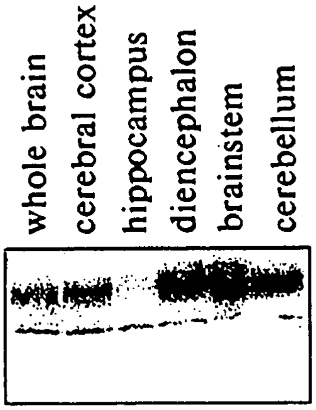
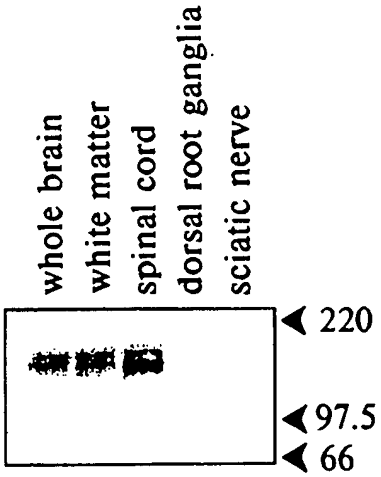


FIG. 30B



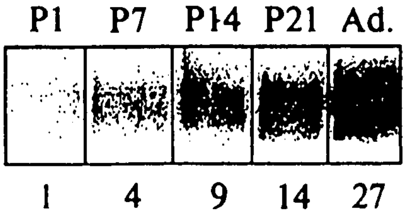


FIG. 30C

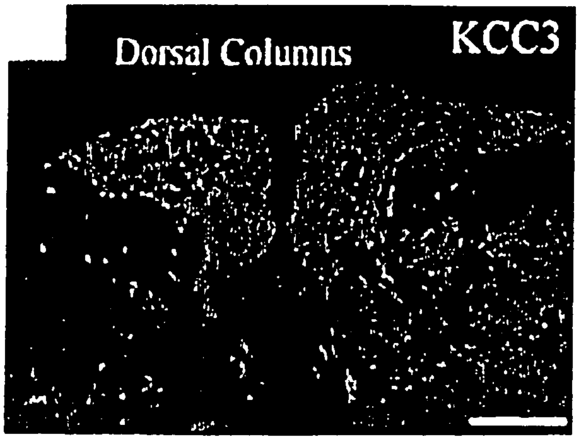


FIG. 31A

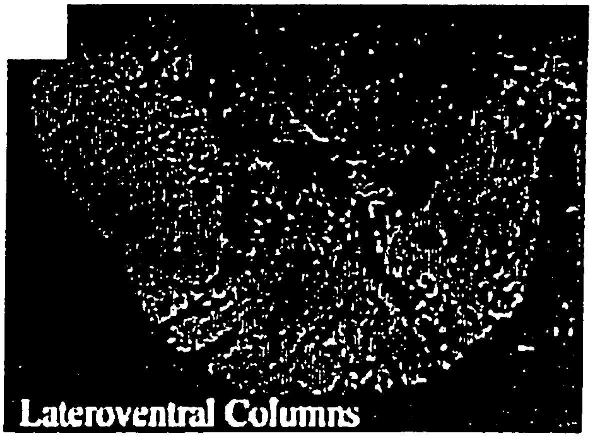


FIG. 31D

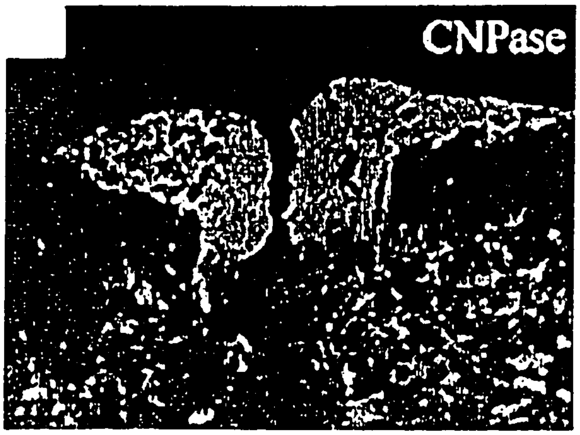


FIG. 31B

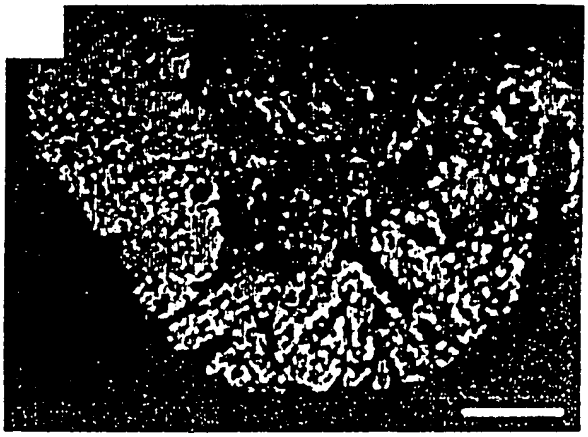


FIG. 31E

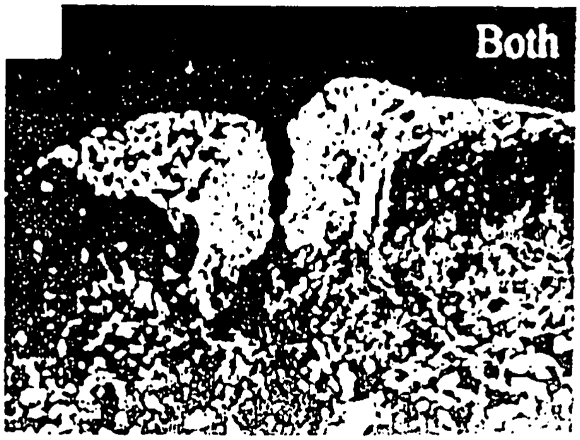


FIG. 31C

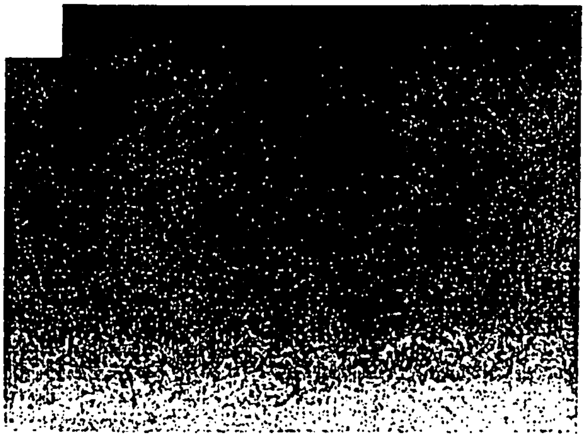


FIG. 31F

Title: Purified and Isolated Potassium-Chloride Cotransporter Nucleic Acids and Polypeptides and Therapeutic and Screening Methods Using Same
Appl[illegible]s): Mount et al.
Serial No.: 09/835,976

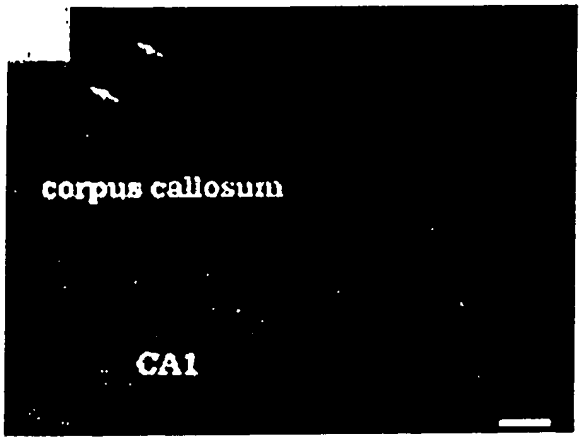


FIG. 32A

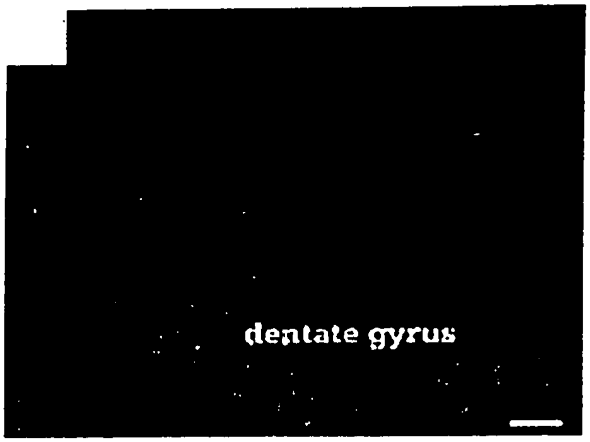


FIG. 32D

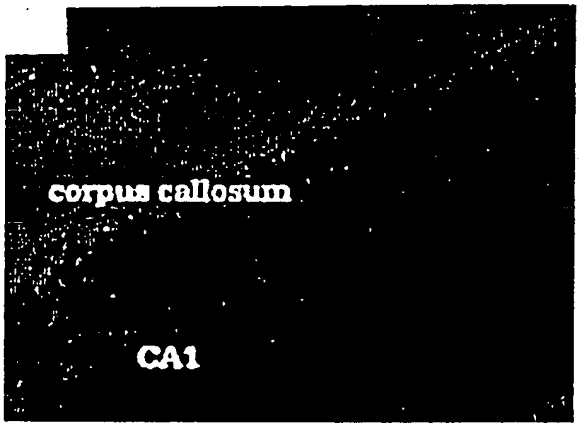


FIG. 32B

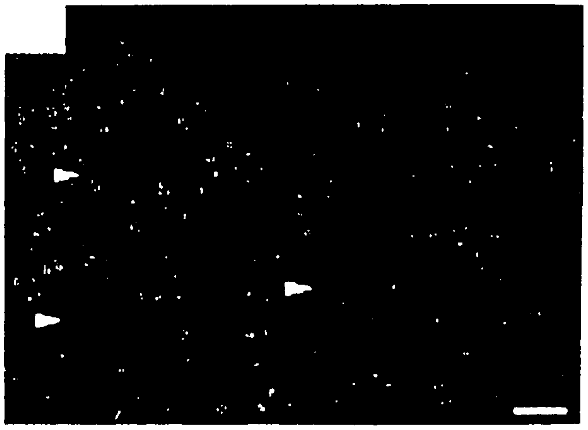


FIG. 32E

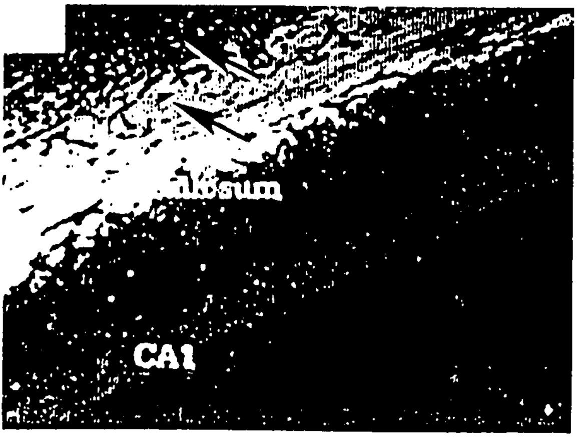


FIG. 32C

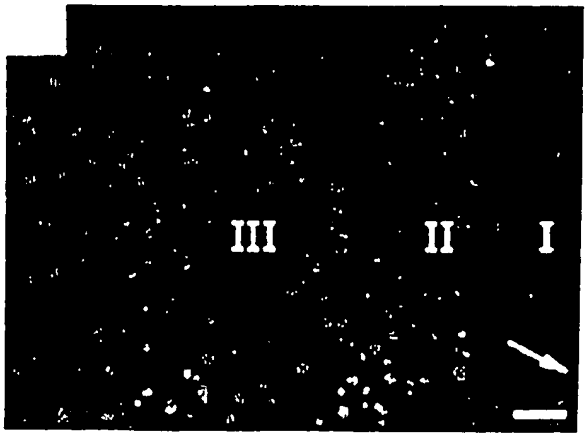


FIG. 32F

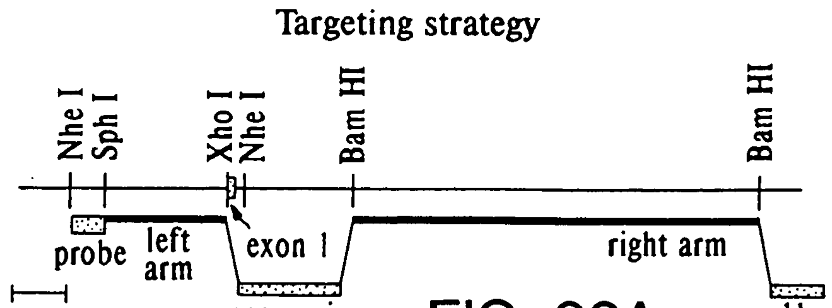


FIG. 33A

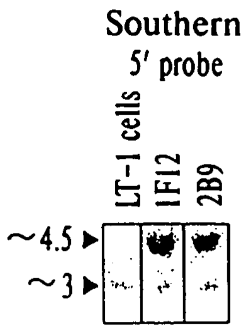


FIG. 33B

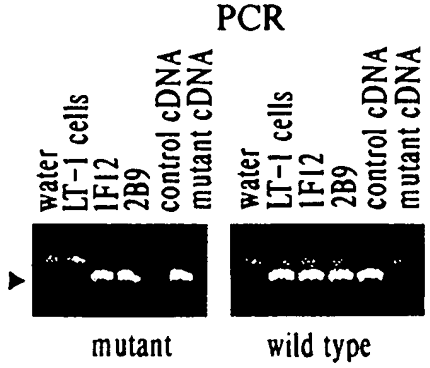


FIG. 33C

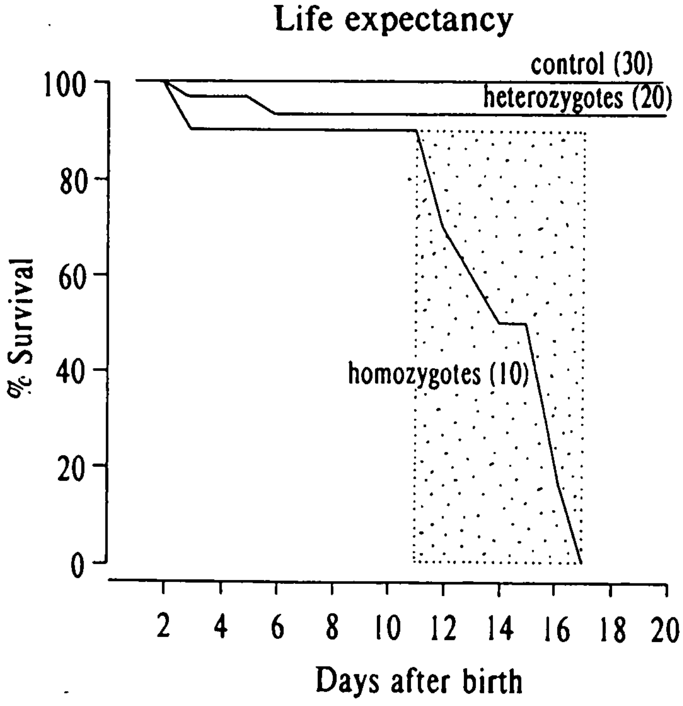


FIG. 33D

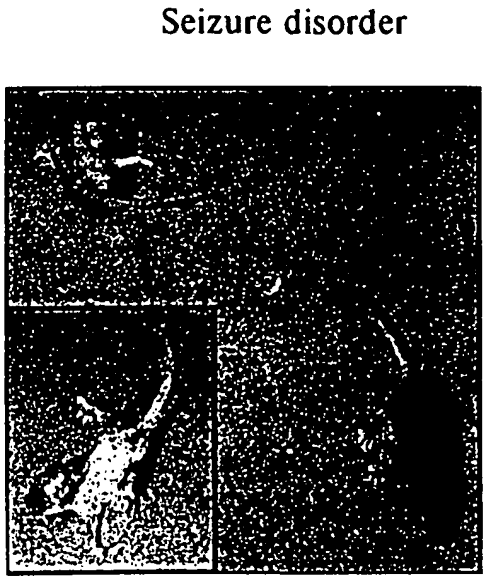


FIG. 33E

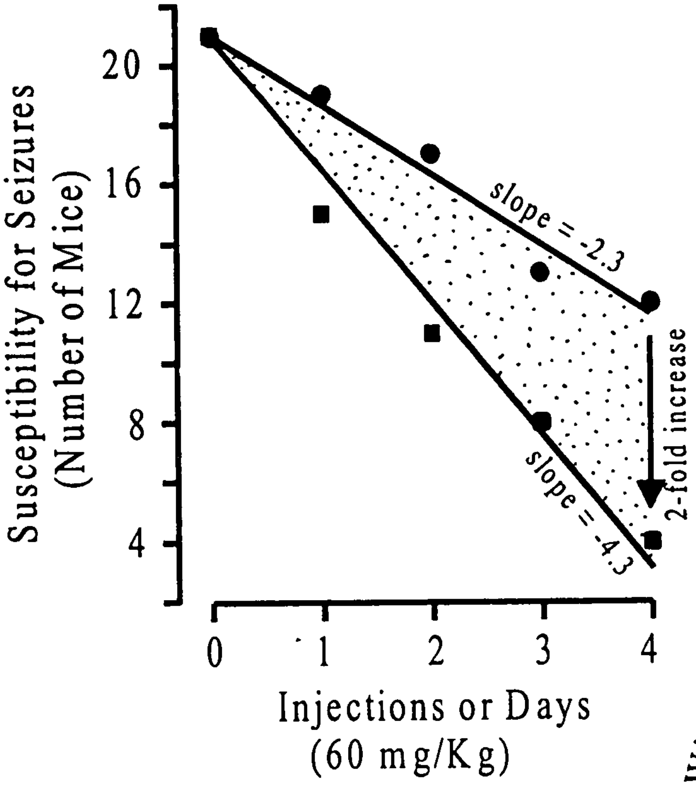


FIG. 34A

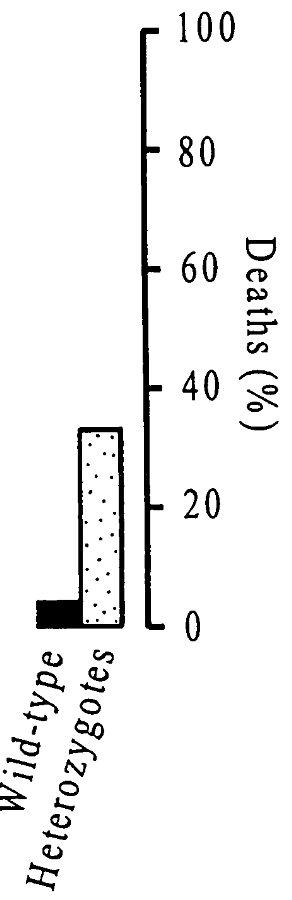


FIG. 34B

KCC3 Construct

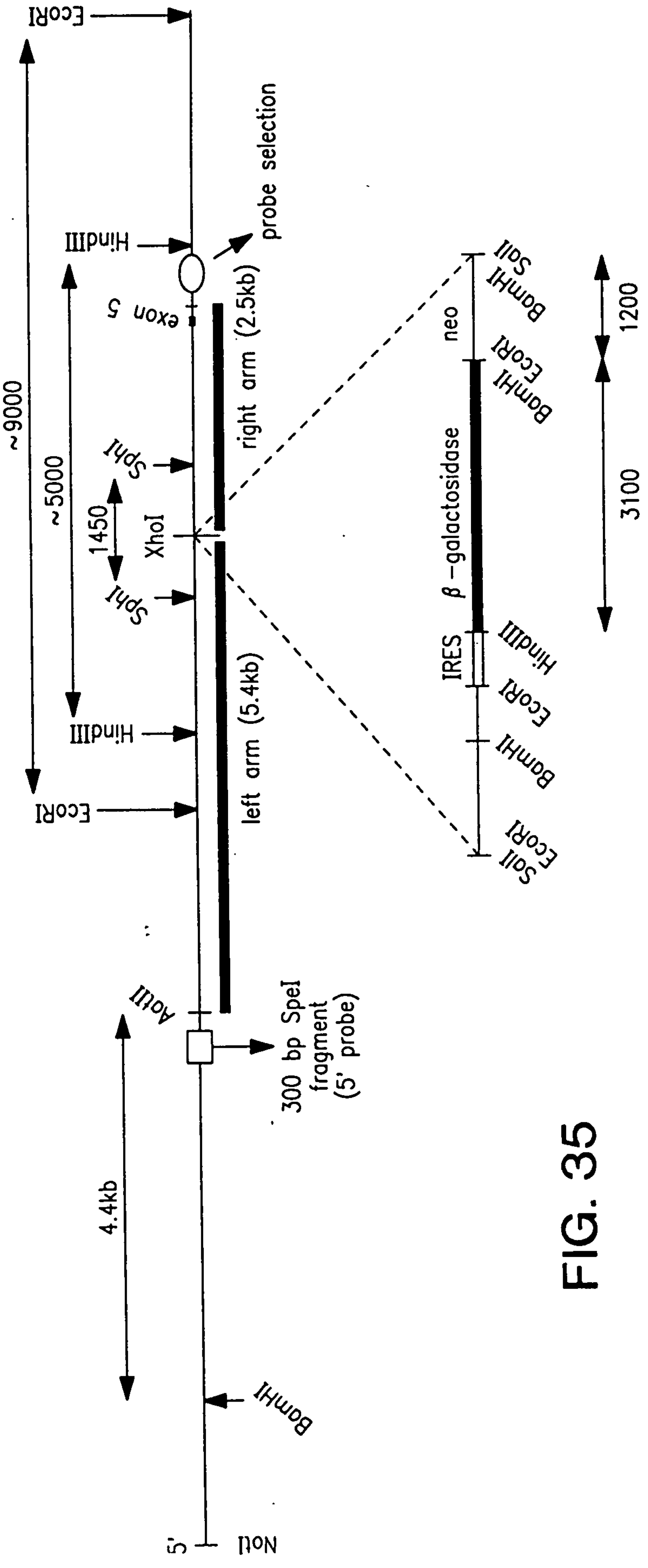


FIG. 35

Title: Purified and Isolated Potassium-Chloride Cotransporter ··cleic Acids and Polypeptides and Therapeutic and ;eening Methods Using Same
Appl): Mount et al.
Serial No.: 09/835,976

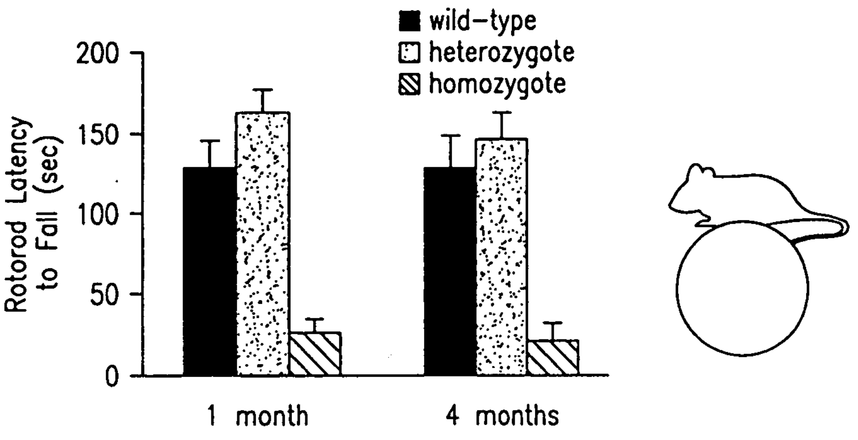


FIG. 36A

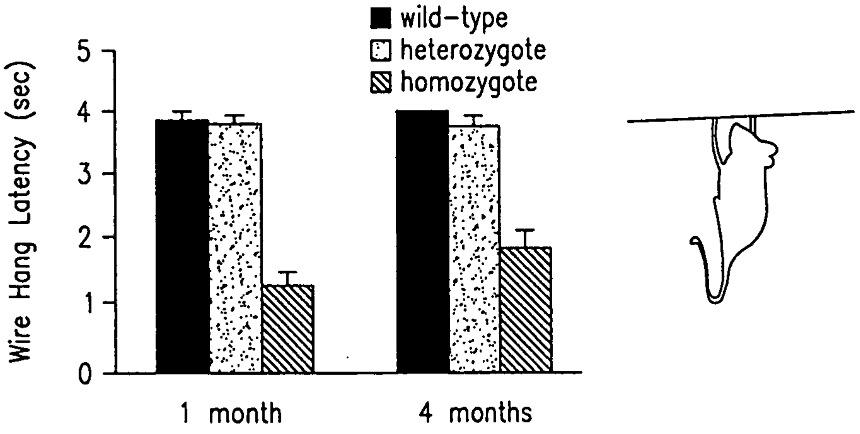


FIG. 36B

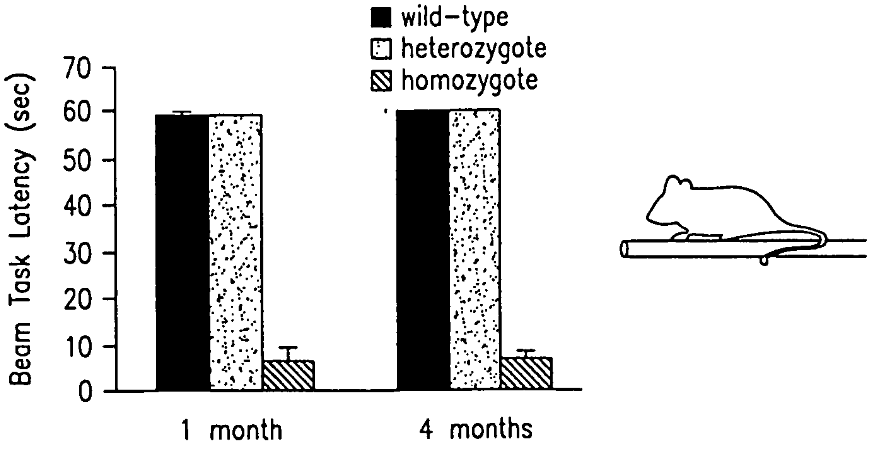


FIG. 36C

Title: Purified and Isolated Potassium-Chloride Cotransporter [illegible]cleic Acids and Polypeptides and Therapeut[illegible]d [illegible]creening Methods Using Same
Appli[illegible](s): Mount et al.
Serial No.: 09/835,976

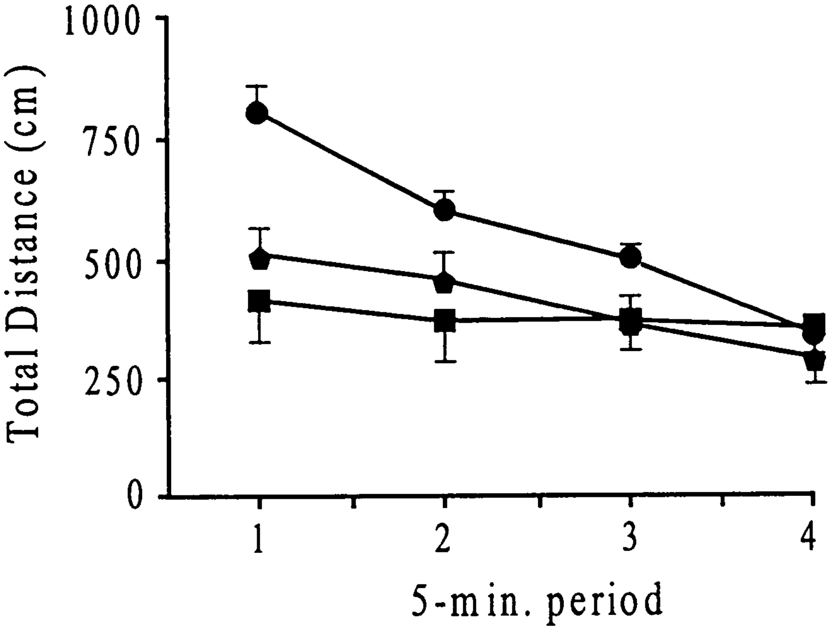


FIG. 37A

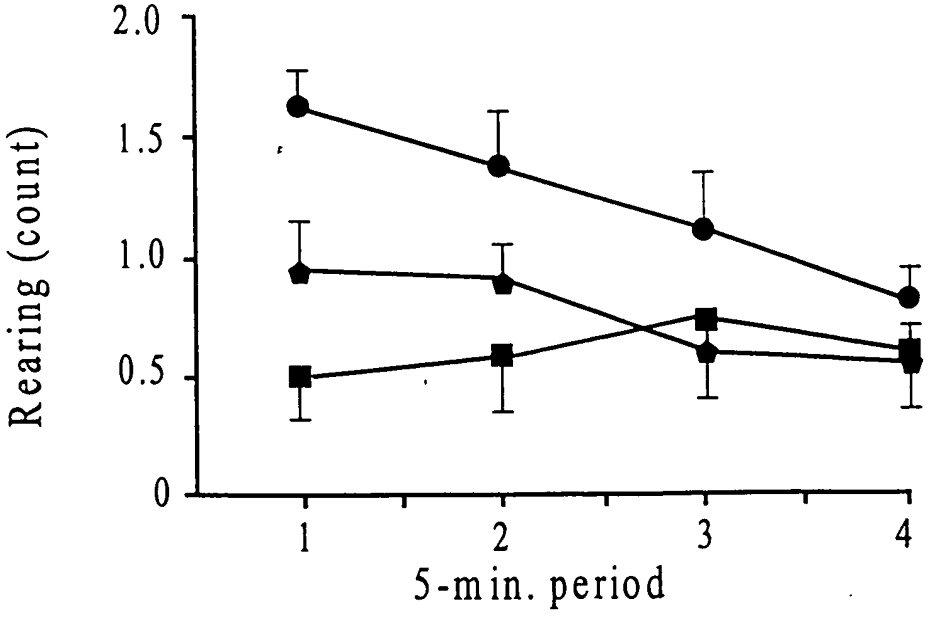


FIG. 37B

Title: Purified and Isolated Potassium-Chloride Cotransporter Nu[illegible] Acids and Polypeptides and Therapeutic and [illegible]ing Methods Using Same
Applicant[illegible] Mount et al.
Serial No.: 09/835,976

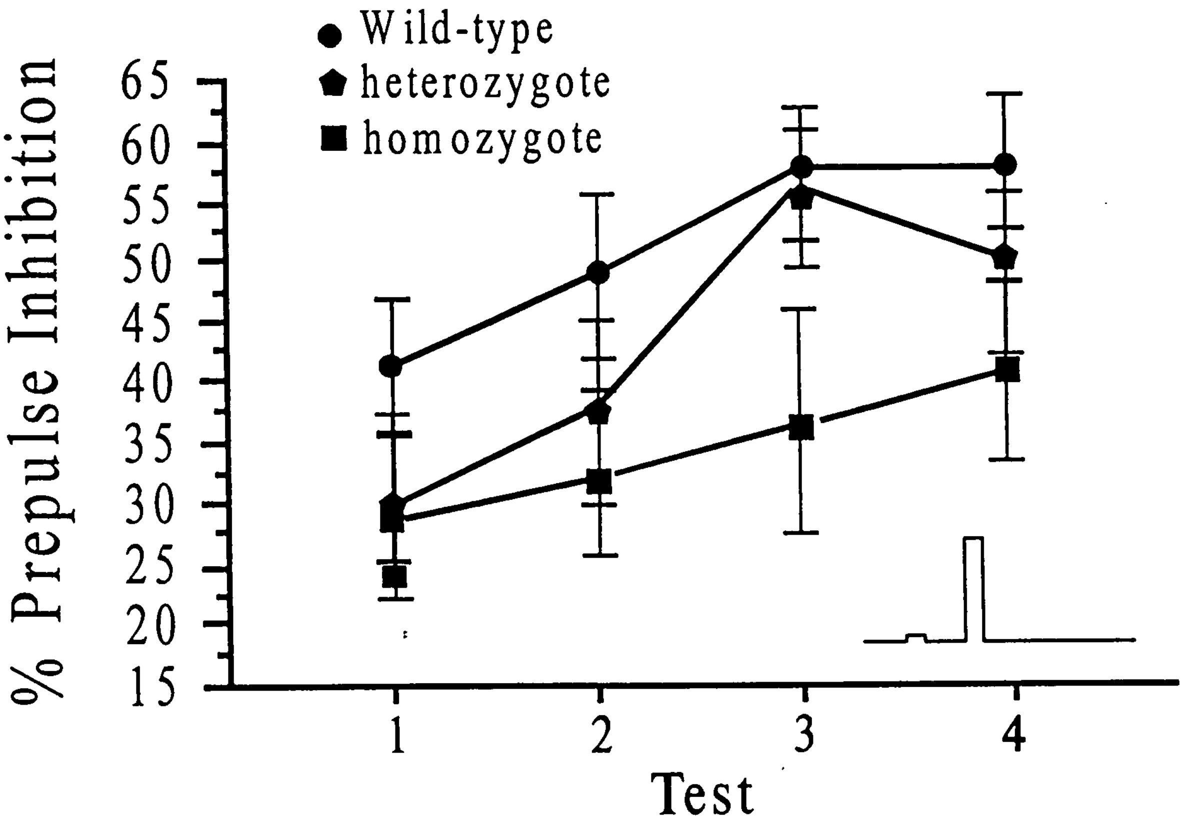


FIG. 38

Title: Purified and Isolated Potassium-Chloride Cotransporter Nucleic Acids and Polypeptides and Therapeutic and [illegible]ening Methods Using Same
Applic[illegible]: Mount et al.
Serial No.: 09/835,976

FIG. 39A

FIG. 39B

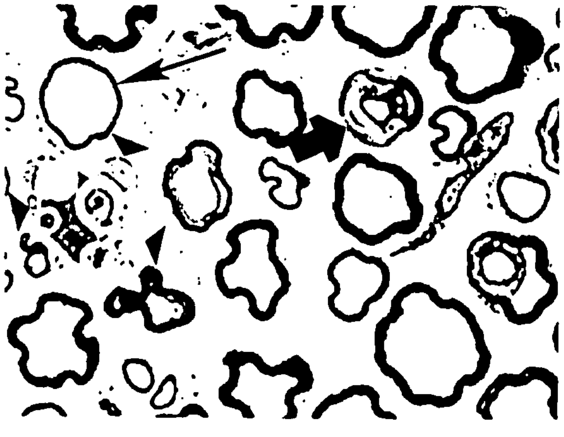


FIG. 39C

FIG. 39D

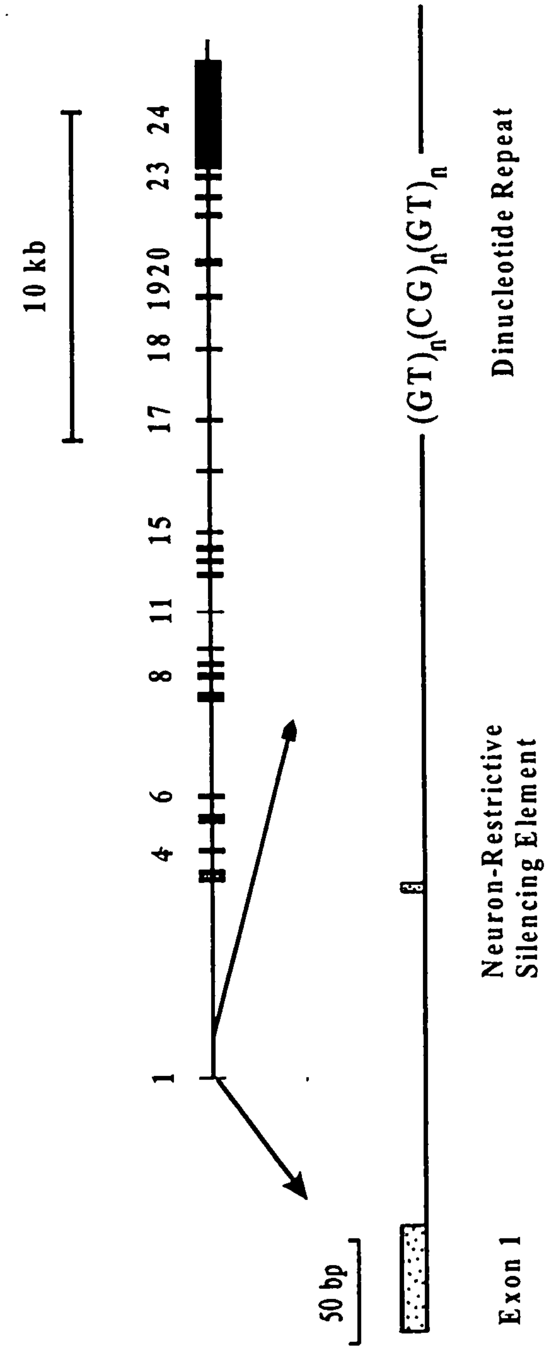

10 kb
1
4
6
8
11
15
17
18
1920
23
24
50 bp
Exon 1
Neuron-Restrictive Silencing Element
$(GT)_n(CG)_n(GT)_n$
Dinucleotide Repeat

FIG. 40

Sequence of the hKCC2 dinucleotide repeat in several individuals:

Sample 1:

Allele A	$(GT)_{18}\,(GC)_{7}\,(AT)_{1}\,(GT)_{4}\,(GC)_{1}\,(GT)_{11}$ / Total = 84
Allele B	$(GT)_{16}\,(GC)_{5}\,(AT)_{1}\,(GT)_{5}\,(GC)_{1}\,(GT)_{9}$ / Total = 74

Sample 2:

Allele A	$(GT)_{18}\,(GC)_{4}\,(AT)_{2}\,(GT)_{4}\,(GC)_{2}\,(GT)_{11}$ / Total = 82

Sample 3:

Allele A	$(GT)_{16}\,(GC)_{6}\,(AT)_{1}\,(GT)_{4}\,(GC)_{1}\,(GT)_{11}$ / Total = 78
Allele B	$(GT)_{14}\,(GC)_{5}\,(AT)_{1}\,(GT)_{4}\,(GC)_{1}\,(GT)_{11}$ / Total = 72

Sample 4:

Allele A	$(GT)_{19}\,(GC)_{6}\,(AT)_{2}\,(GT)_{4}\,(GC)_{2}\,(GT)_{10}$ / Total = 86
Allele B	$(GT)_{17}\,(GC)_{7}\,(AT)_{2}\,(GT)_{4}\,(GC)_{2}\,(GT)_{10}$ / Total = 84

Sample 5:

Allele A	$(GT)_{17}\,(GC)_{6}\,(AT)_{2}\,(GT)_{4}\,(GC)_{1}\,(GT)_{10}$ / Total = 80
Allele B	$(GT)_{16}\,(GC)_{6}\,(AT)_{2}\,(GT)_{3}\,(GC)_{2}\,(GT)_{10}$ / Total = 78

Sample 6:

Allele A	$(GT)_{15}\,(GC)_{6}\,(AT)_{1}\,(GT)_{4}\,(GC)_{1}\,(GT)_{11}$ / Total = 76
Allele B	$(GT)_{16}\,(GC)_{5}\,(GT)_{1}\,(AT)_{1}\,(GT)_{4}\,(GC)_{1}\,(GT)_{11}$ / Total = 78

Sample 7:

Allele A	$(GT)_{16}\,(GC)_{4}\,(GT)_{1}\,(AT)_{1}\,(GT)_{5}\,(GC)_{1}\,(GT)_{10}$ / Total = 76

FIG. 41